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Food fraud in the fisheries and aquaculture sector

Joint FAO/IAEA Centre
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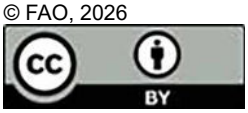

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This report was developed by Esther Garrido Gamarro, Giulia Loi and Christina Vlachou, who wrote the initial draft. The case studies and other specific sections were developed by Alice Giusti, Andrea Armani, Angela Melado, Bert Popping, Daniel Carvalho, Debashish Mazumder, Gabriela Delpiani, Idoia Olabarrieta, Jagoda Crawford, Joachim Molkentin, Juan Martín Díaz de Astarloa, Katja Kaltenbach, Marine Cusa, Michael Bank, Miguel Angel Pardo, Patricia Gadd, Patrick Adams, Peter Psomadakis, Rosalee Hellberg, Sergio Mathias Delpiani, Stefano Mariani and Willette Demian.

The case studies presented in this report were consolidated during a series of expert meetings on food fraud in the fisheries and aquaculture sector and relevant sections were reviewed by Cornelia Boesch, Carmen Bullón and Teemu Viinikainen.

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Abbreviations

BOLD	Barcode of Life Data Systems
COI	cytochrome c oxidase subunit I
DNA	deoxyribonucleic acid
DNTPs	deoxynucleotide triphosphates
ELISA	enzyme linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FBO	food-business operator
FDA	Food and Drug Administration (United States of America)
FINS	forensically informative nucleotide sequencing
GFSI	Global Food Safety Initiative
HACCP	hazard analysis and critical control points
HPLC	high-performance liquid chromatography
HRMA	high-resolution melt analysis
HRMS	high-resolution mass spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
IEF	isoelectric focusing
IFS	International Featured Standards
IUU	illegal, unreported, and unregulated (fishing)
LAMP	loop-mediated isothermal amplification
MPA	marine protected area
MS	mass spectrometry
NGS	next-generation sequencing
NIR	near-infrared
NMR	nuclear magnetic resonance
PAP	polyphenolic adhesive protein
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SNP	single-nucleotide polymorphism
SQF	Safe Quality Food (standard)
TTX	tetrodotoxin
USP	U.S. Pharmacopeia
UV-Vis	ultraviolet-visible (spectroscopy)
XRF	X-ray fluorescence

Executive summary

The global fisheries and aquaculture sector, which produced over 185 million tonnes of aquatic products in 2022 and was valued at USD 195 billion, is becoming increasingly vulnerable to food fraud. This vulnerability arises from the sector's complexity, the wide variety of species traded (over 12 000), and the involvement of multiple inspection authorities across international supply chains. Food fraud in the aquatic sector includes practices such as species substitution, mislabelling, adulteration, counterfeiting and misrepresentation of origin or production method. These actions, often driven by economic motives, pose significant risks to public health, consumer trust and marine conservation.

This report provides a detailed overview of food fraud in the aquatic sector, outlining its types, causes and impacts. It demonstrates that species substitution and mislabelling are the most common forms of fraud, with studies indicating that up to 20 percent of fishery and aquaculture products globally are mislabelled. Fraud is especially prevalent in restaurants and catering services, where visual identification is challenging, and in processed products, where the species identity can be masked. Health risks linked to fraud include exposure to toxins, allergens, pathogens and contaminants, especially when mislabelled products come from unauthorized sources or bypass safety checks.

A series of international case studies illustrates the extent and consequences of food fraud in the aquatic sector and provides an overview of the most common cases and the available tools to fight food fraud in the sector.

Regulatory frameworks and standards play a vital role in fighting fraud in the aquatic sector. The report reviews international standards, including Codex Alimentarius, FAO guidelines, and GFSI-benchmarked schemes (such as BRCGS, FSSC 22000, International Featured Standards, and Safe Quality Food), as well as national laws in Australia, Canada, the United States of America and the European Union. It advocates for harmonized labelling requirements, the mandatory inclusion of scientific names, and better traceability systems. Raising consumer awareness and increasing industry transparency are also highlighted as critical steps to reduce fraud and support sustainable practices in the aquatic sector.

The report underscores the importance of DNA-based methods such as barcoding, polymerase chain reaction and next-generation sequencing for precise species identification, particularly in processed or mixed-seafood products. It also examines protein-based, nuclear and spectroscopic techniques, including enzyme-linked immunosorbent assay, high-performance liquid chromatography, matrix-assisted laser desorption ionization–time-of-flight mass spectrometry, stable isotope analysis, infrared spectroscopy and nuclear magnetic resonance, used to detect fraud and verify provenance. In addition, the report describes innovative methods such as portable X-ray fluorescence and machine-learning models, which are emerging as tools for rapid origin verification.

In summary, food fraud in the aquatic sector is a widespread and complex issue with serious health, economic and environmental consequences. Combating it requires a coordinated effort involving strict enforcement, advanced analytical tools, stakeholder collaboration and public education. This report offers practical recommendations to bolster global efforts to ensure authenticity, safety and integrity in aquatic products.

CHAPTER 1
Background

In 2018, the Food and Agriculture Organization of the United Nations (FAO) published the FAO Fisheries and Aquaculture circular *Overview of food fraud in the fisheries sector* (FAO, 2018a). This document highlights that the fisheries and aquaculture sector is one of the food sectors most subject to fraud. This is due to the complexity of the sector, the perishability of aquatic products and consumer demand, which is increasingly oriented towards processed products, which are more difficult to identify. The 2018 circular emphasizes some of the consequences of fraud for the aquatic sector and stresses the importance of legislative instruments and Codex Alimentarius texts. Building on this effort, FAO decided to develop a report highlighting the most common forms of fraud in the fisheries and aquaculture sector and the possible food-safety implications. For this purpose, FAO and the Joint FAO/IAEA Centre of Nuclear Techniqes in Food and Agriculture convened experts from various disciplines to participate in developing the report and to contribute case studies that illustrate some of the most common types of fish fraud, their incidence and the public-health impact, as well as tools to prevent and fight food fraud in the sector. This report also discusses the economic incentive of fraud in fisheries and aquaculture, the methods currently used to test fishery products, and the current food-safety requirements in force in some countries.

Since 2018, FAO has published several additional reports on food fraud. A relevant resource is the report *International and National Regulatory Strategies to Counter Food Fraud* (FAO, 2022), which introduces the available international regulatory guidance and the potential legal strategies at national and regional levels. The report identifies and analyses some of the regulatory approaches to food fraud that countries have chosen and considers the role of the private sector in food-fraud regulation. Another relevant report is the document, *Food fraud – Intention, detection and management* (FAO, 2021), which describes the key aspects of food fraud and discusses a set of measures that food-safety authorities can take to stop this persistent problem. Lastly, it is important to mention that Codex Alimentarius is preparing guidelines on the prevention and control of food fraud to guide relevant competent authorities and food-business operators in detecting, preventing, mitigating and controlling food fraud, to help protect the health of consumers and to ensure fair practices in food trade (including regarding feed for food-producing animals).

CHAPTER 2
Introduction

The production of fishery and aquaculture products reached 185.4 million tonnes in 2022, with 89 percent used for human consumption, resulting in an estimated per capita consumption of almost 21 kg. More than 230 countries and territories participated in the international trade of aquatic products, reaching a record value of USD 195 billion. The global net trade of aquatic animal products in low- and middle-income countries reached USD 45 billion, which is greater than that of all other agricultural products combined (FAO, 2024a). The volume and value of production and the complex trade flows make the sector especially vulnerable to fraud. Other factors that increase the sector's vulnerability to fraud are the great number of aquatic species (12 413 individual species are included in the list of Aquatic Sciences and Fisheries Information System [ASFIS]) and lack of knowledge of species taxonomy; the particularities of the aquatic production sector, where wild-capture fisheries represent 49 percent of production; and the numerous competent authorities involved in fisheries inspection and food control for aquatic products who must coordinate in order to cover all the necessary aspects to ensure product safety and authenticity.

Food fraud is defined as a deliberate practice intended to deceive others regarding the prescribed specifications or expected characteristics of food (as established by national regulations or agreed standards and norms), to gain an unfair economic advantage.

The consequences of food fraud are significant: consumers can be misled into purchasing products that are unsafe or different (or of lower quality) than advertised, which can harm their health. Apart from affecting consumer health, food fraud negatively impacts consumer trust in the food industry and in responsible authorities.

Academia and institutions take into consideration the following classification for food fraud in the fisheries and aquaculture sector:

- **Adulteration:** Adulteration implies the addition of a non-authentic or fraudulent substance to the final product; for instance, adding colouring agents, water, or other fillers to aquatic products. An example could be the addition of additives to tuna to make it look fresher or change the colour of the flesh. The fraudulent component may lead to illness.
- **Counterfeit**: Counterfeit food fraud occurs when all aspects of an original high-value aquatic product are replicated in a fraudulent food product, and it is packaged to make it look like the original.
- **Simulation**: Simulation involves creating a product that resembles a high-value aquatic commodity without being an exact copy. In other words, it is the creation of a fake version of the original aquatic commodity. An example is the imitation of crab meat with surimi or similar; that is, using deboned, washed fish flesh mixed with additives (usually coming from lean fish species) to simulate crab meat.
- **Diversion:** Diversion is the sale or distribution of legitimate products outside of their intended markets. An example is importing fishery products into a specific nation from a country that is not authorized to export to this nation.
- **Misbranding**: Misbranding is providing false or misleading information on packaging, such as incorrect claims about sustainability or organic certification. (This can happen with both wild-capture fishery products and farmed products.)
- **Overrun:** Overrun happens when producers do not follow production agreements

and produce a legitimate product in excess of established limits. Normally, these products are sold outside the regulated supply chain. An example of this is overfishing of certain species (beyond the agreed quota) and selling the excess production outside the regulated channels.

- **Species substitution**: Species substitution involves replacing a high-value species with a cheaper one for economic gain. An example of this is selling farmed salmon as wild-caught or substituting red snapper (*Lutjanus campechanus*) with tilapia. When a fishery product is not captured according to established agreements, and the quota is exceeded, a reverse substitution can occur; that is, selling the illegally caught product as a similar product, even at a lower price, to facilitate commercialization. An example could be commercializing illegally landed Atlantic bluefin tuna (*Thunnus thynnus*) as yellowfin (*Thunnus albacares*) or bigeye tuna (*Thunnus obesus*).
- **Tampering and mislabelling:** Tampering and mislabelling occur when a legitimate product and packaging are fraudulently used. An example is changing the labelling or the information about the expiry date of the product. In the case of fisheries and aquaculture, mislabelling can include misrepresenting the origin or method of production, such as labelling farmed fish as wild caught.
- **Theft:** Theft occurs when a product is stolen and passed off as if it were legitimately procured. Stolen products are distributed outside regulated or controlled supply chains.

Fishery and aquaculture products are particularly vulnerable to fraud. According to Marvin *et al.* (2016), the probability of fraud in this sector is estimated at 20.6 percent, significantly higher than that of meat (13.4 percent) and fruits and vegetables (10.4 percent). The most frequently reported types of fraud are species substitution and mislabelling, though the underlying causes of mislabelling are often diverse and context dependent (Donlan and Luque, 2019). Substitution typically involves two aquatic species: the expected species, according to the label, and the substitute, which is the actual species sold. A global study by Oceana (2016) found that one in five of over 25 000 seafood samples tested were mislabelled under the name of other species, with fraud occurring at every stage of the value chain. Cases were reported in 55 countries across all continents except Antarctica, with hake, escolar and Asian catfish among the most substituted species. Alarmingly, 58 percent of substitutions involved species that pose health risks to consumers.

Further studies reinforce the scale of the issue:

- In restaurants, misdescription rates are significantly higher than in retail settings, with 30 percent of seafood products mislabelled (Pardo, Jiménez and Pérez-Villarreal, 2016).
- In Peru, seafood fraud was detected in 43 percent of samples, with particularly high rates in ceviche (78 percent) and sashimi (28 percent) (Velez-Zuazo *et al.,* 2021).
- In the European Union, one-third of mass caterers served mislabelled seafood, with pangasius frequently used as a substitute (Pardo *et al.,* 2018).
- In China, 75.5 percent of tested seafood products were identified as species outside the expected family (Xiong *et al.,* 2019).

As reflected in the numerous studies, food fraud in the fisheries and aquaculture sector presents unique challenges compared to other food systems. The sector's specific characteristics, such as species diversity, complex supply chains and processing practices, require tailored approaches to prevent and control fraud. This report outlines common food-fraud issues in fisheries and aquaculture and highlights some

of the most relevant analytical techniques to detect fraud, especially for some fraud categories such as species substitution. It offers information, guidance and tools to help public institutions and the private sector, including competent authorities, academic institutions, fish companies and fish workers, better understand and address these challenges and, by doing so, build trust and protect consumers.

CHAPTER 3
Food fraud in the aquatic sector: a potential risk to food safety

In the complex and globalized world of fisheries and aquaculture, food fraud has emerged as a silent but significant threat to public health. While the motivations behind fraudulent practices may vary, including economic gain and market manipulation, the consequences often converge on one critical issue: food-safety vulnerability.

Aquatic products are particularly susceptible to various forms of fraud due to their high value, perishability, and the complexity of their supply chains. From adulteration with unauthorized substances to species substitution and mislabelling, each category of fraud carries distinct risks that can compromise consumer health, erode trust in the industry, and undermine regulatory systems.

This chapter explores the different types of food fraud relevant to fisheries and aquaculture, highlighting their implications for food safety. Through concrete examples and analysis, it aims to raise awareness regarding how fraudulent practices not only deceive consumers but expose them to serious health hazards.

Understanding these risks is essential for developing effective prevention strategies and ensuring the integrity of aquatic food products.

3.1 ADULTERATION

There is a wide array of substances that may be added to fishery and aquaculture products to alter their appearance, weight, or perceived freshness. Not all adulterants pose direct food-safety risks. For example, water, ice glaze, or other fillers are sometimes added to increase product weight. While these practices primarily affect organoleptic properties (such as texture and flavour) and economic value, they may not always compromise consumer health. However, they still constitute fraud when not properly declared.

More concerning are adulterants that alter the visual appearance of fish products, especially when unauthorized or used in excess. A notable example is the use of carbon monoxide (CO) to enhance the red colour of fish flesh, particularly in species like tuna. While CO treatment can make fish appear fresher, its use is banned or restricted in many jurisdictions as it is considered deceptive and carries potential health risks. Similarly, synthetic dyes and colouring agents may be added to mimic the natural coloration of premium species, which can mislead buyers and, in some cases, introduce toxic compounds.

Other documented adulteration practices that affect food safety include:

- the use of preservatives or chemicals (such as formaldehyde) to mask spoilage;
- the addition of flavour enhancers or masking agents to disguise off-flavours in lower-quality fish.

These practices not only deceive consumers but can undermine food safety, distort market competition, and erode trust in the fisheries and aquaculture sector. Effective monitoring, clear labelling and enforcement of food-safety regulations are essential to combat adulteration and protect public health.

3.2 COUNTERFEIT AND SIMULATION

High-value aquatic products are particularly vulnerable to counterfeit and simulation fraud, where products are intentionally designed to imitate premium aquatic commodities. These practices not only deceive consumers but also pose risks by bypassing inspections and safety protocols that would normally apply to the genuine product.

In the case of counterfeit, the replication of branded or certified aquatic commodities, including the misuse of labels such as "wild-caught," "organic," or those claiming sustainable fishing or farming practices, might imply the use of fake documentation or packaging that mimic legitimate sources, making it difficult for consumers and inspectors to detect the deception and avoiding certain food-safety checks that would have been necessary for the real commodity.

In the case of simulation, the creation of a product that resembles a high-value commodity might imply the application of processes and the use of ingredients that are not declared. A common example is the imitation of crab meat using surimi, a processed fish paste often made from species such as Alaska pollock. While surimi is a legitimate product when properly labelled, it becomes fraudulent when marketed as real crab meat. This can be problematic, especially when undeclared additives such as egg white, soy, or artificial colouring agents are used, which may trigger allergic reactions or sensitivities in consumers.

Other examples of counterfeit and simulation include:

- imitation shrimp or scallops made from moulded fish paste or starch-based compounds;
- simulated roe made from seaweed extract or gelatine, sold as caviar.

These practices can undermine traceability, food safety and consumer trust, especially when the substitute product is of lower nutritional value or contains undeclared allergens. Regulatory frameworks and inspection protocols must be adapted to detect and prevent both counterfeit and simulated aquatic products, particularly in complex international supply chains.

3.3 DIVERSION

When aquatic products are sold or distributed outside their intended markets, the product can be legitimate, but depending on the origin of the product, food safety might be compromised. For instance, fishery and aquaculture products coming from a country that is not authorized to export to the importing country due to not meeting the requirements to do so, can bring with them issues that were not detected in their country of origin. These products may not meet the importing country's minimum sanitary, environmental, or traceability standards, and any hazards present, such as contaminants, pathogens, or undeclared allergens, may go undetected due to differences in inspection protocols.

Diversion can also occur through the re-routing of products intended for industrial or animal-feed use into the human food chain, or through the misuse of quota-exempt or restricted species. In such cases, the product may not be subject to the same level of scrutiny, and its safety for human consumption may be questionable.

These practices undermine consumer protection, market integrity and international trade agreements, and require the coordinated effort of regulatory authorities, customs agencies and industry stakeholders to detect and prevent.

3.4 MISBRANDING

The provision of false or misleading information on packaging, such as incorrect claims about sustainability or organic certification, can become a food-safety issue.

A common form of misbranding involves incorrect claims about sustainability, origin, or organic certification. For instance, aquaculture products labelled as "organic"

may in fact originate from farms that do not meet the required standards. If such products contain elevated levels of antimicrobials, veterinary drugs, or chemical residues, they can pose serious health risks to consumers, especially when these substances are not declared or exceed legal limits.

Other examples of misbranding include:

- misuse of eco-labels or certification logos, without proper verification or licensing;
- false claims of geographical origin, such as labelling farmed shrimp from Southeast Asia as "Mediterranean" or "local";
- misleading freshness indicators, such as "fresh" labels on previously frozen products.

Misbranding, as well as mislabelling, can also obscure traceability, making it difficult to track the product's journey through the supply chain. This is particularly problematic in cases of food recalls, contamination events, or illegal fishing activities, where accurate labelling is essential for public health and regulatory enforcement.

To combat misbranding, robust labelling regulations, verification systems and consumer education are critical. Technologies such as blockchain traceability, QR-code tracking, and DNA barcoding are increasingly being used to ensure label accuracy and product authenticity.

3.5 OVERRUN

When producers do not follow production agreements such as quotas or harvest volumes, often in violation of regulatory frameworks, this can lead to food-safety issues, even when the product itself may be legitimate in origin. A common example is when fishers capture more than the authorized quota, and the excess catch is sold through informal or unregulated channels. These diverted products often bypass official sanitary inspections, meaning they might not be tested for contaminants, pathogens, or spoilage – posing risks to consumer health.

Overrun can also occur in aquaculture operations, where producers exceed stocking densities or harvest volumes beyond those permitted under their license. This can lead to:

- increased disease pressure due to overcrowding, which can be a food-safety problem in terms of zoonotic diseases;
- higher use of veterinary drugs or antimicrobials, which may not be properly monitored or declared;
- environmental degradation, which can indirectly affect product safety and quality.

In both wild capture and aquaculture, overrun undermines resource sustainability, market fairness and consumer protection. It also complicates traceability systems, making it difficult to verify the origin, handling and safety of the product.

Moreover, overrun practices can mask illegal, unreported and unregulated (IUU) fishing, where excess catch is intentionally hidden from authorities. This not only violates conservation efforts but also increases the risk of food fraud, especially when such products are mislabelled or mixed with legal catch.

To address overrun, it is essential to strengthen:

- catch documentation schemes and electronic monitoring systems;
- aquaculture-production reporting and inspection of operations;
- cross-border cooperation to detect and prevent unauthorized trade flows.

3.6 SPECIES SUBSTITUTION

Species substitution is one of the most prevalent forms of food fraud in the fishery and aquaculture sector. It involves replacing one declared aquatic species with another,

often one of lower value, more abundant, or less regulated. This practice is primarily driven by economic incentives and can have serious food-safety implications.

One major concern is that the substitute species may carry food-safety hazards that require specific handling, preparation, or consumption restrictions. For example:

- Puffer fish (fugu) contains tetrodotoxin, a potent neurotoxin, and must be prepared by licensed professionals. Substituting this species without proper handling can be fatal.
- Some fish species are not intended to be consumed raw due to their microbiological profile or parasite load. When substituted and served as sushi, sashimi, ceviche, or other raw preparations, they can pose risks and cause a variety of illnesses such as anisakiasis, listeriosis and vibriosis.

In addition to health risks, species substitution undermines consumer trust, traceability and sustainability efforts. For instance, substituting endangered or overfished species with unregulated species can distort conservation data.

Detection of species substitution often requires DNA barcoding or molecular techniques. Strengthening traceability systems, enforcing labelling regulations and increasing awareness among consumers and stakeholders in aquatic value chains are essential to mitigate the risks associated with species substitution.

3.7 MISLABELLING

When packaging is fraudulent, consumers can receive misleading information about a variety of aspects such as the species, ingredients, nutritional composition or expiration date, as well as claims related to sustainability or origin. In all cases, there can be implications for human health, such as:

- Expired products that are relabelled with extended shelf-life dates may be consumed past their safe period, increasing the risk of microbial contamination or spoilage-related illnesses.
- Undeclared ingredients, such as allergens (including shellfish, soy, gluten, or egg proteins), can trigger severe allergic reactions in sensitive individuals.
- Mislabelling species can result in the consumption of fish species with a different intended use (for example, to be consumed only cooked, not raw; or to be avoided by certain population groups).

To combat mislabelling, robust labelling regulations, traceability systems and enforcement mechanisms are essential. Technologies such as blockchain, digital-traceability platforms, and DNA-based species identification are increasingly being used to verify product authenticity and ensure accurate labelling throughout the supply chain.

3.8 THEFT

When a fishery or aquaculture product is stolen and passed off as if it were legitimately procured, these commodities often bypass official sanitary inspections, meaning they are not subject to the same controls for microbiological hazards, chemical residues, or temperature abuse during transport and storage. This increases the likelihood of the product being unfit for consumption, especially in cases where cold-chain integrity is compromised or where the product originates from restricted harvest areas.

Examples of common theft-related food fraud include:

- theft of high-value species (such as abalone or sea cucumber) from harvesting areas, which are then sold through black markets or misrepresented in formal supply chains;
- theft of bivalve molluscs from closed or contaminated areas, which can result in the distribution of products containing marine biotoxins or pathogens such as *Vibrio* spp., leading to serious illnesses such as paralytic shellfish poisoning (PSP) or amnesic shellfish poisoning (ASP).

Addressing theft in the seafood sector requires:

- strengthened monitoring and surveillance at harvest sites, ports and processing facilities;
- enhanced traceability systems to verify the origin and legality of products;
- coordinated enforcement between fishery authorities, customs and food-safety agencies.

CHAPTER 4
Economic incentive

Food fraud is commonly associated with an economic benefit for fraudsters. In the case of wild-capture fisheries, the bargaining power between aquatic food dealers and vessel owners has been shown to play an important role in the incentive to mislabel. Vessel owners typically have less bargaining power due to the perishability of the products and limited alternatives in terms of market sale for buyers willing to accept the risk of purchasing fraudulent products. Dealer-harvesters (vertically integrated companies) present the strongest incentives to misreport (DePiper and Holzer, 2024).

Overall, the average price differential (and variability) varies from species to species and changes over time. There are studies pointing out that the mislabelling price differentials of substitute species range from + EUR 25 to - EUR 12 (approximately + USD 28 to - USD 13), with sturgeon caviar having the greatest price differential of substitute species among a large range of studied species. The same study showed a substantial profit (EUR 10, approximately USD 11) for yellowfin tuna when used as a substitute for Atlantic bluefin and bigeye tuna. Similarly, Atlantic salmon (*Salmo salar*) labelled as Pacific salmon captures an average profit of EUR 9 (approximately USD 10) (Donlan and Luque, 2019).

Food fraud creates a lot of uncertainty in consumers, and there are studies that show buyers are willing to pay more for a product that grants authenticity and provides information on the traceability of fisheries and aquaculture products. For example, one study analysed buyers' willingness to pay a premium of £0.79 (approximately USD 0.98) for a portion of authentic pollan fillets with respect to a portion of herring fillets. The same study points to a premium of 7.1 percent to 16.7 percent for a portion of fish fillets (250g) that are more likely to be authentic compared to other products for which food fraud is more likely to occur (McCallum *et al.*, 2022). The results of the studies show that there is an incentive for food businesses to ensure traceability and authenticity. This can be done through a number of strategies, such as certification, or by setting specific traceability requirements.

Adulteration of food is also motivated by financial advantage. A common economically motivated adulteration is the undeclared substitution of ingredients in a product, and there are studies that point to aquatic commodities as the main food category subject to incidents of this nature, although the specific price differential has not yet been described (Everstine, Spink and Kennedy, 2013).

The substitution of wild-captured aquatic species with farmed aquatic species can have an economic incentive as well. For instance, in the United States, the price for farmed species such as salmon can range from USD 4.35 to USD 4.90 per fish sold fresh, for specimens between 10 pounds and 18 pounds, while the price of wild salmon can range from GBP 5.5 to GBP 7 (USD 7.23 to USD 9.21) for specimens weighing the same (Urner Barry, 2025). There are also differences for products like seabass and sea-bream, not only due to the different production methods (wild caught versus farmed). European seabass (*Dicentrarchus labrax*) provides an interesting example of the price differentials that exist for the same species of fish depending on production method and country of origin. Supplies of wild-caught European seabass are limited

and represent just 2 percent of the total commercialized, with the remaining 98 percent being sourced from aquaculture. In December 2024, imported, farmed seabass weighing 400 g to 600 g from Greece and Türkiye were selling on the Roman wholesale market for EUR 6.80/kg and EUR 4.20/kg, respectively. By comparison, Italian farmed fish of the same size averaged EUR 12.50/ kg, close to double the price of Greek fish and triple that of fish of Turkish origin (FAO, 2025a).

CHAPTER 5
Taxonomy and nomenclature as tools to prevent fraud

Worldwide, more than 36 000 species of finfish have been described. The amount of information required to distinguish among them is not always available to fishery workers due to a lack of up-to-date taxonomic guides at the country, regional and global levels. As a result, the practice of grouping species into larger categories for statistical purposes, as well as the misidentification of species, have become one of the most serious handicaps in the collection of fishery data by species. (This is especially the case in tropical and subtropical regions, where high biodiversity, combined with generally limited taxonomic capacity and resources, increase the complexity of species identification.) In addition, the increasing globalization of fishery products introduces new challenges to the identification of aquatic organisms.

An effective mitigation strategy against fraud in fisheries and aquaculture requires integrated action. Such a strategy should include an official list of commercial fish names cross-referenced to scientific nomenclature. The list would help reduce the taxonomic ambiguities that enable intentional misreporting. Additionally, this nomenclature base must be supported by mandatory labelling rules that oblige operators to disclose, at a minimum, the scientific and commercial name, production method, catch or farming area and other traceability elements, thereby ensuring that verifiable information accompanies aquatic foods from landing to final sale.

FAO provides global-level species catalogues as well as regional and field species-identification guides through the FAO FishFinder Species Identification and Data Programme and recently also through the EAF-Nansen Programme, which improves fisheries management across Africa and the Bay of Bengal. Likewise, online resources, such as FishBase (an information system that provides data on the biology of all fish) and Eschmeyer's Catalog of Fishes (an authoritative reference for taxonomic fish names) offer guidance in resolving issues regarding the correct scientific name for species (FAO, 2013).

The use of proper nomenclature is crucial for accurate species identification. The FAO Fisheries and Aquaculture Division collates global capture and aquaculture production statistics by species, where available, or alternatively at genus, family, or higher taxonomic levels. The Aquatic Sciences and Fisheries Information System (ASFIS) list for fishery statistics represents the standard taxonomic reference system for this collection. The list is updated and released annually, with new species items added to accommodate new production data or in response to requests from national authorities and international organizations. Currently, the ASFIS list includes 13 708 records, of which 3 901 are statistical categories used in FAO statistics (according to the data released in March 2025). Common names, when available, are also provided in English, French, Spanish, Arabic, Chinese and Russian, and the list indicates whether each species is included in the FAO global capture and aquaculture production datasets.

The FAO tools mentioned above, particularly the FishFinder programme and the ASFIS list, play a crucial role in combating food fraud, more specifically fish-species substitution at the beginning of the value chain, where accurate species identification

is most critical. However, taxonomic nomenclature is dynamic, with species names and classifications continuously revised as scientific understanding evolves, requiring these tools to be updated regularly to maintain their effectiveness. When properly maintained, these tools help establish a transparent and verifiable foundation for aquatic food products from the moment they are landed or harvested. By improving species identification and standardizing nomenclature, they strengthen traceability systems and support regulatory frameworks aimed at preventing food fraud. Moreover, they empower national authorities and industry stakeholders to make informed decisions, enforce labelling compliance, and maintain consumer trust in seafood products.

CHAPTER 6
Standards and norms

Norms, requirements and standards form the basis that define whether a product is acceptable or not and are key tools for fighting food fraud in the fisheries and aquaculture sector. Standards and legal instruments provide useful guidance for national governments in combatting food fraud. There is great variability in regulatory approaches to fight food fraud, and implementing and enforcing an optimal legal approach requires thoughtful analysis and design (Roberts, Viinikainen and Bullon, 2022). The recently drafted Codex Alimentarius *Guidelines on the Prevention and Control of Food Fraud* (currently under review) will supplement existing Codex texts, all of which constitute the international framework for national strategies and the benchmark global standards to combat food fraud.

6.1 CODEX ALIMENTARIUS STANDARDS

A range of international organizations have processes for setting standards regarding food fraud. Aspects related to food fraud are already addressed in many existing Codex texts, such as the General Standard for the Labelling of Prepackaged Foods (CXS 1-1985), the General Standard for the Labelling of Food Additives when Sold as Such (CXS 107-1981), the Principles for Traceability/Product Tracing as a Tool within a Food Inspection and Certification System (CXG 60-2006), the Codex Code of Ethics for International Trade in Food including Concessional and Food Aid Transactions (CXC 20-1979), the Principles and Guidelines of National Food Control Systems (CXG 82-2013), the Guidelines for Design, Production, Issuance and Use of Generic Official Certificates (CXG 38-2001), and the Principles and guidelines for the exchange of information between importing and exporting countries to support the trade in food (CXG 89-2016).

In addition, as indicated, Codex Alimentarius is currently working on guidelines on the prevention and control of food fraud. The purpose of this work is to provide guidance to competent food-safety authorities and food-business operators on the detection, prevention, mitigation and control of food fraud to help protect consumer health and ensure fair practices in food trade, including feed for food-producing animals. This guidance is intended to support or supplement existing Codex texts by providing additional guidance specific to food fraud that can be considered within national food-control systems.

6.2 PRIVATE FOOD-SAFETY STANDARDS

There are a number of what are commonly referred to as private-law schemes. Private-law schemes are not part of a country's regulatory system but often form the basis of agreements between trading partners, such as food-business operators. Many of these schemes have published standards for different aspects related to the food supply chain, such as food manufacturing, transportation and brokerage. These standards, although not focusing explicitly on food fraud, contain elements to combat it. This chapter will focus primarily on the standards related to food manufacturing and how food fraud is captured within their schemes. Several of these food-quality and safety standards have existed for a number of years. SQF, for example, developed by the Safe Quality Food Institute, was developed in Australia in 1994, and FSSC 22000, developed by the non-profit organization Foundation FSSC, was developed in 2009.

However, these food standards differ, and if a food manufacturer's client asked for two different certifications, significant resources were required for the manufacturer to obtain them both.

This is where the Global Food Safety Initiative (GFSI) comes into play. Founded in the year 2000, it aimed to harmonize, to a certain degree, the major food-safety-related standards, enabling them to be mutually acceptable. This was achieved by benchmarking the standards against certain criteria. In 2007, seven major food retailers agreed to reduce duplication in the supply chain through the common acceptance of any of the GFSI-benchmarked schemes. Carrefour, Tesco, Metro, Migros, Ahold, Walmart and Delhaize paved the way to achieving GFSI's vision of "once certified, accepted everywhere" (SGS, 2014). Version 7.1 introduced two new scopes of benchmarking: one for food fraud and one for food defence, requiring all GFSI-recognized certification programmes to include vulnerability assessments and mitigation plans. As a consequence, all GFSI-benchmarked schemes amended the relevant section in their standards.

A number of private-law food-safety schemes are GFSI benchmarked. These cover not only food but also aquaculture and agriculture. At present, thirteen schemes are benchmarked (GFSI, 2023):

- BRCGS (formerly known as BRC)
- Canada GAP
- Equitable Food Initiative (EFI)
- Freshcare
- FSSC 22000
- Global Red Meat Standard (GRMS)
- Global Seafood Alliance
- Global Gap
- International Featured Standards (IFS)
- Japan Food Safety Management Association (JFSM)
- ASIAGAP
- PrimusGFS
- SQF

The most widely used schemes are BRCGS, FSSC 22000, IFS and SQF. SQF is almost exclusively used in the United States, with few certified sites outside the country. In contrast, FSSC 22000 is an international standard with currently more than 32 000 certificates in Asia, Europe, North America, Latin America and the Caribbean. IFS and BRCGS are also international standards, but they are more dominant in specific regions: BRCGS in the United Kingdom of Great Britain and Northern Ireland, and IFS in most European countries.

In 2014, in addition to other important topics, GFSI focused on food fraud in the supply chain. It published numerous documents, covering GFSI's position on mitigating the public-health risk of food fraud (GFSI, 2014) and tackling food fraud through food-safety management systems (GFSI, 2019).

The major difference between the various schemes is the level of prescriptiveness of the standards. While FSSC 22000 and SQF are standards that state what is required, they generally provide little detail on how this must be achieved. On the other hand, BRCGS and IFS are much more detailed regarding what is required from each of the certified sites. In addition, the standards have different conditions for fail/pass and for minor non-compliance, non-compliance and critical non-compliance. A detailed comparison can be found in the SGS document, *Comparing Global Food Safety Initiative (GFSI) Recognised Standards* (SGS, 2014).

6.2.1 Sections on food fraud and traceability in GFSI-benchmarked schemes: BRCGS, FSSC 22000, IFS and SQF

This section evaluates the commonalities of the most used standards (BRCGS, FSSC 22000, IFS and SQF) related to food fraud and traceability.

6.2.1.1 BRCGS

Food fraud

In the current version of this standard, version 9 (BRCGS, 2022a), several sections mention food fraud:

Section 2, The Food Safety Plan – HACCP, indicates the following in clause 2.7.1, which deals with food manufacturers having to list all hazards for each process step:

> The HACCP food safety team shall identify and record all the potential hazards that are reasonably expected to occur at each step in relation to product, process, and facilities. This shall include hazards present in raw materials, those introduced during the process or surviving the process steps, and consideration of the following types of hazards: …. fraud (e.g., substitution or deliberate/ intentional adulteration… (BRCGS, 2022A, p. 25.

Section 3.4 is categorized as fundamental and deals with internal audits. It requires food manufacturers to have their food-defence and food-fraud prevention plans audited.

Food-fraud vulnerability assessment under BRCGS also includes the risk assessment for raw materials received from suppliers for potential substitution or food fraud (Section 3.5.1.1).

This standard also requires food manufacturers to have a system in place to minimize the risk of purchasing fraudulent or adulterated food raw materials (Section 5.4, with detailed requirements in subsections 5.4.1 and 5.4.2).

In addition, Section 9.2.1 (p. 89) reads "The company shall have a documented supplier approval procedure which identifies the process for initial and ongoing approval of suppliers and the manufacturer/processor of each product traded". This requirement covers the potential for adulteration or fraud.

Traceability

The BRGCS standard mentions two types of traceability: supply-chain traceability and the traceability of ingredients and products within the manufacturing site.

Under the food-safety and management-system section (Section 3), subsection 3.9 is dedicated to traceability of raw materials through all stages of processing. This section is marked "fundamental". This section deals with the traceability of products **within** the manufacturing site.

For supply-chain traceability, the entire Section 9.6 of the BRCGS standard is dedicated to it. However, at present, the standard only requires one-up, one-down traceability. This means the company is only required to keep records of the companies it purchased products from and the companies it sold products to. Further traceability is not required under this section.

In addition to the actual standard, the interpretation guideline for BRCGS Global Standard Food Safety Issue 9 (BRCGS, 2022b) contains useful additional information in the sections related to food fraud, food defence and traceability.

6.2.1.2 IFS

Food fraud

In the IFS food standard, version 8, the key section dedicated to food fraud is Section 4.20. Two major points are addressed in this section: food-fraud-vulnerability assessment and the development of a mitigation plan. (Section numbers with an asterisk (*) indicate mandatory actions and requirements.) (IFS, 2023, p. 76):

> 4.20 Food fraud
>
> 4.20.1 The responsibilities for a food fraud vulnerability assessment and mitigation plan shall be defined. The responsible person(s) shall have the appropriate specific knowledge.
>
> 4.20.2(*) A documented food fraud vulnerability assessment, including assessment criteria, shall be documented, implemented, and maintained. The scope of the assessment shall cover all raw materials, ingredients, packaging materials, and outsourced processes, to determine the risks of fraudulent activity in relation to substitution, mislabelling, adulteration or counterfeiting.
>
> 4.20.3 A food fraud mitigation plan shall be documented, implemented and maintained with reference to the vulnerability assessment, and shall include the testing and monitoring methods.
>
> 4.20.4(*) The food fraud vulnerability assessment shall be reviewed, at least once within a 12-month period or whenever significant changes occur. If necessary, the food fraud mitigation plan shall be revised/updated accordingly.

In addition, the glossary also defines each of the terms and provides additional useful information, such as the minimum criteria for food-fraud-vulnerability assessment. In other standards, this relevant information is provided either as part of the standard text itself or as a separate guidance document.

Traceability

Traceability requirements are described in Section 4.18. Failing to comply with traceability requirement 4.18.1 is a so-called "knock-out" (KO) criterion, leading to the failure of the audit. The section also specifies the aspects that need to be addressed, such as mass-balance checks. In the IFS food standard, in-factory and supply-chain traceability are dealt with in the same section, which is different from the BRCGS standard. Section 4.18 states (IFS, 2023, p. 72):

> 4.18 Traceability
>
> 4.18.1(*) KO N° 7: A traceability system shall be documented, implemented, and maintained that enables the identification of product lots and their relation to batches of raw materials, and food contact packaging materials, and/or materials carrying legal and/or relevant food safety information. The traceability system shall incorporate all relevant records of:
>
> - receipt
> - processing at all steps
> - use of rework
> - distribution.
>
> Traceability shall be ensured and documented until delivery to the customer.
>
> 4.18.2(*) The traceability system, including mass balance, shall be tested at least once within a 12-month period or whenever significant changes occur. The test samples shall reflect the complexity of the company's product range. The test records shall demonstrate upstream and downstream traceability (from delivered products to raw materials, and vice versa).
>
> 4.18.3 The traceability from the finished products to the raw materials and to the customers shall be performed within four (4) hours maximum. Test results, including the timeframe for obtaining the information, shall be recorded and,

where necessary, actions shall be taken. Time-frame objectives shall be aligned with customer requirements, if less than four (4) hours are required.
4.18.4 Labelling of semi-finished or finished product lots shall be made at the time when the goods are directly packed to ensure clear traceability of goods. Where goods are labelled later, the temporarily stored goods shall have a specific lot labelling. Shelf life (e.g., best before date) of labelled goods shall be defined using the original production batch.
4.18.5 If required by the customer, identified representative samples of the manufacturing lot or batch number shall be stored appropriately and kept until expiration of the "Use by" or "Best before" date of the finished products and, if necessary, for a determined period beyond this date.

6.2.1.3 FSSC 22000

Food fraud

In the FSSC 22000 standard, in version 6 (FSSC 22000, 2023), food fraud and food defence, as well as the requirement for mitigation measures, are addressed in several sections.

Food-fraud mitigation and vulnerability assessment are addressed in Section 2.5.4 (FSSC 22000, 2023, p 72):

2.5.4 FOOD FRAUD MITIGATION (ALL FOOD CHAIN CATEGORIES)
2.5.4.1 VULNERABILITY ASSESSMENT
The organization shall:

a) Conduct and document the food fraud vulnerability assessment, based on a defined methodology, to identify and assess potential vulnerabilities; and
b) Develop and implement appropriate mitigation measures for significant vulnerabilities. The assessment shall cover the processes and products within the scope of the organization.

2.5.4.2 PLAN

a) The organization shall have a documented food fraud mitigation plan, based on the output of the vulnerability assessment, specifying the mitigation measures and verification procedures.
b) The food fraud mitigation plan shall be implemented and supported by the organization's FSMS.
c) The plan shall comply with the applicable legislation, cover the processes and products within the scope of the organization, and be kept up to date.
d) For food chain category FII, in addition to the above, the organization shall ensure that its suppliers have a food fraud mitigation plan in place.

While this section is comparably short, the FSSC has published two additional guidance documents detailing relevant information on food-fraud mitigation (FSSC 22000, 2019a) and food defence (FSSC 22000, 2019b).

Traceability

Unlike the BRCGS and IFS standards, no major section of the FSSC standard is dedicated to traceability. However, traceability is mentioned in sections 2.5.2., 4.3 and 5.1.1.

6.2.1.4 SQF

SQF has different codes for food production and manufacturing segments, including Primary Plant Production, Primary Animal Production, Aquaculture, Food Manufacturing, Pet Food Manufacturing, Animal Feed Manufacturing and Animal Product Manufacturing, to name but a few. This section focuses food fraud and traceability in the Quality Code, the Food Manufacturing Code and the Aquaculture Code.

Food fraud in the Quality Code

The SQF Quality Code, Edition 9 (SQF, 2022a), includes Section 2.7 dedicated to food fraud. It states (p. 52) :

> 2.7 2 Food Defense and Food Fraud
> 2.7.1 Food Defense Plan (Mandatory)
> 2.7.1.1. A food defence threat assessment shall be conducted to identify potential threats that can be caused by a deliberate act of sabotage or terrorist-like incident.
> 2.7.1.2 A food defence plan shall be documented, implemented, and maintained based on the threat assessment (refer to 2.7.1.1).
> 2.7.2 Food Fraud (Mandatory)
> 2.7.2.1 The methods, responsibility, and criteria for identifying the site's vulnerability to food fraud, including susceptibility to raw material or ingredient substitution, finished product mislabeling, dilution, or counterfeiting, shall be documented, implemented, and maintained.
> 2.7.2.2 A food fraud mitigation plan shall be developed and implemented that specifies the methods by which the identified food fraud vulnerabilities shall be controlled, including identified food safety vulnerabilities of ingredients and materials.

Traceability in the Quality Code

Traceability is dealt with in Section 2.6 of the Quality Code (p. 50).

> 2.6 Product Traceability and Crisis Management
> 2.6.2 Product Trace (Mandatory)
> 2.6.3 Product Withdrawal and Recall (Mandatory)

Internal and external traceability requirements are dealt with in the same section, and traceability requirements, as in the BRCGS standard, are one-up, one-down.

Food fraud in the Food Manufacturing Code

Section 2.7 of the SQF Manufacturing Code (SQF, 2022b) provides a more detailed treatment of food fraud than the corresponding clauses in the other standards, defining requirements for both vulnerability assessment and mitigation (p. 52):

> 2.7 Food Defence and Food Fraud
> 2.7.1 Food Defence Plan (Mandatory)
> 2.7.1.1 A food defence threat assessment shall be conducted to identify potential threats that can be caused by a deliberate act of sabotage or a terrorist-like incident.
> 2.7.1.2 A food defence plan shall be documented, implemented, and maintained based on the threat assessment (refer to 2.7.1.1). The food defence plan shall meet legislative requirements as applicable and shall include at a minimum:
> i) The methods, responsibility, and criteria for preventing food adulteration caused by a deliberate act of sabotage or terrorist-like incident;
> ii) The name of the senior site management person responsible for food defence;
> iii) The methods implemented to ensure only authorized personnel have access to production equipment and vehicles, manufacturing, and storage areas through designated access points;
> iv) The methods implemented to protect sensitive processing points from intentional adulteration;

v) The measures taken to ensure the secure receipt and storage of raw materials, ingredients, packaging, equipment, and hazardous chemicals to protect them from deliberate acts of sabotage or terrorist-like incidents;
vi) The measures implemented to ensure raw materials, ingredients, packaging (including labels), work-in-progress, process inputs, and finished products are held under secure storage and transportation conditions; and
vii) The methods implemented to record and control access to the premises by site personnel, contractors, and visitors.

2.7.1.3 Instruction shall be provided to all relevant staff on the effective implementation of the food defence plan (refer to 2.9.2.1).

2.7.1.4 The food defence threat assessment and prevention plan shall be reviewed and tested at least annually or when the threat level, as defined in the threat assessment, changes. Records of reviews and tests of the food defence plan shall be maintained.

2.7.2 Food Fraud (Mandatory)

2.7.2.1 The methods, responsibility, and criteria for identifying the site's vulnerability to food fraud, including susceptibility to raw material or ingredient substitution, finished product mislabelling, dilution, or counterfeiting, shall be documented, implemented, and maintained.

2.7.2.2 A food fraud mitigation plan shall be developed and implemented that specifies the methods by which the identified food fraud vulnerabilities shall be controlled, including identified food safety vulnerabilities of ingredients and materials.

2.7.2.3 Instruction shall be provided to all relevant staff on the effective implementation of the food fraud mitigation plan (refer to 2.9.2.1).

2.7.2.4 The food fraud vulnerability assessment and mitigation plan shall be reviewed and verified at least annually with gaps and corrective actions documented. Records of reviews shall be maintained.

Traceability in the Food Manufacturing Code

Section 2.6 in the Food Manufacturing Code covers traceability more extensively than in the Quality Code. It also includes crisis-management planning. The section states (p. 50):

2.6 Product Traceability and Crisis Management

2.6.1 Product Identification (Mandatory)

2.6.1.1 The methods and responsibility for identifying raw materials, ingredients, packaging, work in progress, process inputs, and finished products during all stages of production and storage shall be documented and implemented to ensure:

i) Raw materials, ingredients, packaging, work-in-progress, process inputs, and finished products are clearly identified during all stages of receipt, production, storage, and dispatch; and
ii) Finished product is labelled to the customer specification and/or regulatory requirements.

2.6.1.2 Product start-up, product changeover, and packaging changeover (including label changes) procedures shall be documented and implemented to ensure that the correct product is in the correct package and with the correct label and that the changeover is inspected and approved by an authorized person. Procedures shall be implemented to ensure that label use is reconciled and any inconsistencies investigated and resolved. Product changeover and label reconciliation records shall be maintained.

2.6.2 Product Trace (Mandatory)
2.6.2.1 The responsibility and methods used to trace product shall be documented and implemented to ensure:
i) Finished product is traceable at least one step forward to the customer and at least one step back from the process to the manufacturing supplier;
ii) The receipt dates of raw materials, ingredients, food contact packaging and materials, and other inputs are recorded (refer to 2.8.1.8 for traceback of allergen containing food products.);
iii) Traceability is maintained where product is reworked (refer to 2.4.6); and
iv) The effectiveness of the product trace system is reviewed at least annually, as part of the product recall and withdrawal review (refer to 2.6.3.2).

Records of raw and packaging material receipt and use and finished product dispatch and destination shall be maintained.
2.6.3 Product Withdrawal and Recall (Mandatory)
2.6.3.1 The responsibility and methods used to withdraw or recall a product shall be documented and implemented. The procedure shall:
i) Identify those responsible for initiating, managing, and investigating a product withdrawal or recall;
ii) Describe the management procedures to be implemented, including sources of legal, regulatory, and expert advice, and essential traceability information;
iii) Outline a communication plan to inform site personnel, customers, consumers, authorities, and other essential bodies in a timely manner about the nature of the incident; and
iv) Ensure that SQFI, the certification body, and the appropriate regulatory authority are listed as essential organizations and notified in instances of a food safety incident of a public nature or product recall for any reason.

2.6.3.2 The product withdrawal and recall system shall be reviewed, tested, and verified as effective at least annually. Testing shall include incoming materials (minimum traceability one step back) and finished product (minimum traceability one step forward). Testing shall be carried out on products from different shifts and for materials (including bulk materials) that are used across a range of products and/or products that are shipped to a wide range of customers.
2.6.3.3 Records shall be maintained of withdrawal and recall tests, root cause investigations into actual withdrawals and recalls, and corrective and preventative actions applied.
2.6.3.4 SQFI and the certification body shall be notified in writing within twenty-four (24) hours upon identification of a food safety event that requires public notification. SQFI shall be notified at foodsafetycrisis@sqfi.com.
2.6.4 Crisis Management Planning
2.6.4.1 A crisis management plan based on the understanding of known potential dangers (e.g., flood, drought, fire, tsunami, or other severe weather events, warfare or civil unrest, computer outage, pandemic, loss of electricity or refrigeration, ammonia leak, labour strike) that can impact the site's ability to deliver safe food shall be documented by senior management, outlining the methods and responsibility the site shall implement to cope with such a business crisis. The crisis management plan shall include, at a minimum:
i) A senior manager responsible for decision making, oversight, and initiating actions arising from a crisis management incident;
ii) The nomination and training of a crisis management team;
iii) The controls implemented to ensure any responses do not compromise product safety;
iv) The measures to isolate and identify product affected by a response to a crisis;

v) The measures taken to verify the acceptability of food prior to release;
vi) The preparation and maintenance of a current crisis alert contact list, including supply chain customers;
vii) Sources of legal and expert advice; and
viii) The responsibility for internal communications and communicating with authorities, external organizations, and media.

2.6.4.2 The crisis management plan shall be reviewed, tested, and verified at least annually with gaps and appropriate corrective actions documented. Records of reviews of the crisis management plan shall be maintained.

Food fraud in the Aquaculture Code

Also, here, the section dealing with food fraud is Section 2.7 (SQF, 2020). The section states (p. 51):

2.7 Food Defence and Food Fraud

2.7.1 Food Defence Plan (Mandatory)

2.7.1.1 A food/product defence threat assessment shall be conducted to identify potential threats as a result of a deliberate act of sabotage or terrorist-like incident.

2.7.1.2 A food defence plan shall be documented, implemented, and maintained based on the threat assessment (refer to 2.7.1.1). The food defence plan shall meet legislative requirements as applicable and shall include at a minimum:

i) The methods, responsibility, and criteria for preventing food adulteration caused by a deliberate act of sabotage or terrorist-like incident;
ii) The name of the senior site management person responsible for food defence;
iii) The methods implemented to ensure only authorized personnel have access to production equipment, vehicles, and storage areas through designated access points;
iv) The methods implemented to protect sensitive operational points from intentional adulteration;
v) The measures taken to ensure the secure receipt and storage of inputs, equipment, and hazardous chemicals to protect them from deliberate acts of sabotage or terrorist-like incident;
vi) The measures implemented to ensure inputs and products are held under secure storage and transportation conditions; and
vii) The methods implemented to record and control access to the premises by employees, contractors, and visitors.

2.7.1.3 Instruction shall be provided to all relevant staff on the effective implementation of the food defence plan (refer to 2.9.2.1).

2.7.1.4 The food defence threat assessment and prevention plan shall be reviewed and tested at least annually or when the threat level, as defined in the threat assessment, changes. Records of reviews of the food defence plan shall be maintained.

2.7.2 Food Fraud (Mandatory)

2.7.2.1 The methods, responsibility, and criteria for identifying the site's vulnerability to food fraud shall be documented, implemented, and maintained. The food fraud vulnerability assessment shall include the site's susceptibility to product substitution, mislabelling, dilution, and counterfeiting or stolen goods that may adversely impact food safety.

2.7.2.2 A food fraud mitigation plan shall be developed and implemented that specifies the methods by which the identified food fraud vulnerabilities shall be controlled and how the plan is communicated to relevant staff to ensure effective implementation.

2.7.2.3 The food fraud vulnerability assessment and mitigation plan shall be reviewed and verified at least annually, with gaps and corrective actions documented. Records of reviews shall be maintained.

Traceability in the Aquaculture Code

As in the Food Manufacturing Code, traceability is dealt with in Section 2.6 of the Aquaculture Code, which states (p. 49):

2.6 Product Traceability and Crisis Management

2.6.1 Product Identification and Traceability (Mandatory)

2.6.1.1 The methods and responsibilities for the product identification system shall be documented and implemented to ensure:

i) Inputs, work-in-progress, and aquacultural products are clearly identified during all stages of receipt, operations, storage, shipping, and transportation; and
ii) All aquacultural products are identified and/or labelled to customer specification and/or regulatory requirements. Product identification records shall be maintained.

2.6.1.2 The responsibility and methods used to trace the product shall be documented and implemented to ensure:

i) Aquacultural product is traceable to the customer (one up) and provides traceability through the process to the input supplier and date of receipt of inputs, materials, and other inputs (one back);
ii) Traceability is maintained where product is reworked (refer to 2.4.3); and
iii) The effectiveness of the product trace system is reviewed at least annually as part of the product recall and withdrawal review (refer to 2.6.2.1). Records for the receipt and use of agricultural inputs and packaging and for finished product dispatch and destination shall be maintained.

2.6.2 Product Withdrawal and Recall (Mandatory)

2.6.2.1 The responsibility and methods used to withdraw or recall product shall be documented and implemented. The procedure shall:

i) Identify those responsible for initiating, managing, and investigating a product withdrawal or recall;
ii) Describe the procedures to be implemented by site management;
iii) Outline a communication plan to inform customers, consumers, authorities, and other essential bodies in a timely manner appropriate to the nature of the incident;
iv) Describe how the withdrawal and recall system is reviewed, tested, and verified at least annually (mock recall); and
v) Ensure that SQFI, the certification body, and the appropriate regulatory authority are listed as essential organizations and are notified in instances of a food safety incident of a public nature or product recall. Records of all product withdrawals, recalls, and mock recalls shall be maintained.

2.6.2.2 Investigation shall be undertaken to determine the cause of a withdrawal or recall, and details of investigations and any action taken shall be documented and recorded.

2.6.2.3 SQFI and the certification body shall be notified in writing within twenty-four (24) hours upon identification of a food safety event that requires public notification. SQFI shall be notified at foodsafetycrisis@sqfi.com.

2.6.3 Crisis Management Planning

2.6.3.1 The methods and responsibility for execution of a crisis management plan shall be documented and implemented. The plan shall include:

i) A listing of known potential dangers (e.g., hurricanes, low water levels, fire, tsunamis, or other severe weather or global events such as pandemics, warfare, or civil unrest) that can impact the site's ability to deliver safe food;
ii) Designated site management responsible for decision making, oversight, communication, and management of the crisis management plan; and
iii) Control measures to ensure any affected product is identified, isolated, and disposed of appropriately.

2.6.3.2 The crisis management plan shall be reviewed, tested, and verified at least annually, with gaps and appropriate corrective actions documented. Records of reviews of the crisis management plan shall be maintained.

6.2.2 Comparison and outlook

While all GFSI-benchmarked food and aquaculture-related schemes deal with food fraud, food defence and traceability, the level of detail differs significantly between the standards. Also, mitigation-plan requirements differ in the level of detailed requirements. With respect to vulnerability assessments and mitigation plans it is worth noting that several foresight systems have been developed to identify food-fraud issues early. Most of these systems monitor events and news across the globe for keywords and can trigger specific alarms if the commodity in question is affected. A public system is the monthly newsletter of the European Commission (European Commission, 2025). Examples of systems behind paywalls that deliver tailored solutions specific to commodities are SGS Digicomply (SGS, 2023), Horizon Scan (FERA, 2023), and the Food Fraud Database (FoodChain ID, 2023). These foresight systems are not compulsory for any of the standards mentioned above but tend to be viewed favourably by auditors. Which of the standards is best for certification depends on the client's requirements, although GFSI aims to make all benchmarked standards mutually acceptable. And while several major supermarkets have committed to mutually accept any of the GFSI-benchmarked standards, there are clear geographically-based preferences for specific standards. For example, the BRCGS standard is dominant in the United Kingdom, and some UK retailers specifically ask for this certification.

6.2.3 Other standards

In addition to the GFSI-benchmarked standards, several not-for-profit organizations have issued their own guidance on food fraud vulnerability assessment and mitigation. The U.S. Pharmacopeial (USP), through the Food Chemicals Codex, developed a Food Fraud Mitigation Guidance document (USP, 2016). A later scientific paper by Gendel, Popping and Chin (2020) discusses the development and application of this USP approach; the authors were members of the USP Expert Panel involved in preparing the original guidance. In addition to the development of such documents, the USP Food Chemicals Codex has numerous standards for food ingredients and dietary supplements that provide information on analytical methods and parameters to ensure the authenticity of ingredients or supplements, as well as a hazard-classification document (Everstine *et al.*, 2018). Similar documents have been generated by other not-for-profit organizations such as the Association of Official Analytical Collaboration and IFT.

Overall, numerous documents on food fraud and mitigation measures have been developed by different bodies, and their usefulness has been evaluated by the International Life Sciences Institute Europe Food Fraud task force. The evaluation resulted in the publication *Food Inauthenticity: Authority Activities, guidance for food operators, and mitigation tools* (Popping *et al.*, 2022).

6.3 NORMS AND RELEVANT INSTRUMENTS FOR FISH FRAUD

National governments and regional organizations are uniquely positioned to develop, implement and enforce measures to combat food fraud in the fisheries and aquaculture sector. International instruments such as Codex Alimentarius texts and other frameworks, including the FAO *Voluntary Guidelines for Catch Documentation Schemes* (FAO, 2017), the *Agreement on Port State Measures* (FAO, 2024b), the *United Nations Convention on Contracts for the International Sale of Goods* (United Nations, 2010), and the International Institute for the Unification of Private Law's *Principles of International Commercial Contracts* (UNIDROIT, 2016), offer a foundational structure for addressing food-fraud issues associated with fisheries and aquaculture. While some governmental efforts are emerging, specific regulatory actions targeting food fraud remain limited. This is largely due to the complex nature of food fraud (which has hidden impacts), lack of awareness on the risks posed by food fraud, lack of centralized data, globalized and sometimes opaque supply chains, the sophisticated methods of food fraud used, and the economic incentives that drive fraudulent behaviour.

One of the key challenges lies in the difficulty of clearly defining food fraud in the absence of any internationally agreed upon legal definitions of the concept. Often, its elements fall within the grey area between adulteration (foods harmful to health) and misbranding (false representation of a product's nature or quality). This ambiguity can lead to fragmented enforcement and a lack of targeted regulatory focus, given that the regulatory responses to the two different forms of food fraud are generally very different. Adulteration is often criminalized, whereas misbranding may more commonly be considered an administrative offence, meaning that they may be subject to different enforcement regimes.

To effectively regulate food fraud, governments – with the active participation of the private sector - must adopt a dual approach: prevention and enforcement. Preventive strategies, which may need to be prioritized over enforcement after an incident has already occurred, can be embedded across various regulatory domains, from illegal fishing and food safety to consumer protection, and criminal legislation. Nonetheless, prevention alone is insufficient. Detection and enforcement are essential. This includes market inspections, official food controls, and collaboration between public and private sectors. Surveillance and monitoring systems play a critical role in identifying and addressing fraudulent activities.

In 2022, FAO described six strategic approaches to regulating food fraud that should consider the food-safety and quality legal framework, consumer-protection legislation, contract law, criminal-law framework, e-commerce operations, and the role of the private sector (FAO, 2022a). A key part of any of these strategies is assessing food-fraud vulnerability – understanding how and where fraud is likely to occur. Fraud is often seen as a crime of opportunity, where motivated individuals exploit weak controls, technical loopholes and complex supply chains like those of aquatic products. Factors such as economic pressure, business culture and lack of oversight can also influence the likelihood of fraud.

Food safety and quality legal framework

This approach relies on existing laws that aim to protect food safety and quality. It places the main responsibility on food-business operators, who must ensure that their products are safe and meet quality standards, as well as not being fraudulent. This includes keeping proper records, applying control systems like hazard analysis and critical control points (HACCP), and having procedures in place for traceability and product recalls.

Although authorities oversee both food safety and quality, limited resources often mean that food-fraud risks receive less attention compared to direct safety concerns. Food safety authorities may also lack awareness, appropriate training, as well as the equipment necessary to detect fraudulent products and practices, as the tools and methods they use to check for safety may not always be appropriate to also control for the authenticity of the product. To strengthen this framework, new technologies such as blockchain are being explored to improve transparency and traceability across the supply chain.

Food labelling is also a key tool in this strategy, helping consumers make informed choices. However, labelling alone cannot fully prevent fraud schemes. Labelling could be strengthened through third-party certification schemes (for instance, for organic or sustainable seafood) that can help distinguish genuine products from fraudulent ones, but they require strong oversight and may be costly for small producers.

Consumer protection legislation

This approach aims to protect consumers from deceptive practices and ensure they receive accurate and truthful information about the food they purchase. Many types of food fraud, such as false labelling, deceptive marketing, or misrepresentation of product quality, are covered by laws that regulate unfair trade practices.

International guidelines, such as the *United Nations Guidelines for Consumer Protection* (UNCTAD, 2016), support this strategy by promoting fair business practices, access to clear and accurate product information, and protection of consumers' economic interests. The *United Nations Guidelines for Consumer Protection* also specifically calls for action against food adulteration and false or misleading marketing claims, reinforcing the importance of transparency and accountability in food labelling and advertising.

Contract law

Food fraud often involves a violation of contract terms within the supply chain. This happens when a supplier knowingly delivers a product that does not match what was agreed upon, such as a different species, lower quality, or misrepresented origin, with the intent of deceiving the buyer. Such cases can be addressed through domestic contract law, which allows the affected party to take legal action and seek compensation. This highlights the role that the private sector can have – independently of any action from the public sector – in combatting food fraud in their own supply chains. On an international level, legal frameworks like the International Institute for the Unification of Private Law's *Principles of International Commercial Contracts* (UNIDROIT, 2016) also support cancelling contracts if they were based on fraudulent information or failure to disclose important facts. This aligns with the core idea of food fraud as a deliberate act of deception for unfair gain.

Criminal law framework

Food fraud – as the name implies – is fraud, a crime in virtually all criminal laws around the world. This approach treats food fraud – when it fulfils the elements of the crime as established by the law – as a criminal offense, using national criminal laws that define fraud and adulteration to punish and deter offenders. To apply this effectively, it is important to understand the different types of fraudsters, such as insiders, opportunists, or even organized-crime groups, who may be involved in complex schemes.

E-commerce operations

Online food sales present unique challenges that require specific legal and strategic approaches as food operator's responsibilities in food e-commerce operations are often more difficult to identify and enforce. A strong legal framework is essential

to protect consumers buying food online and to prevent fraud in the online setting. One key concern is traceability, as digital supply chains are often complex and spread across multiple regions. Technologies like blockchain are being explored to improve transparency and track products more effectively. Additionally, some authorities use strategies such as "mystery shopper" methods (where the inspector purchases food products online for inspection, without revealing their identity) to conduct official controls for food ordered online.

Role of the private sector

The private sector plays a vital role in addressing food fraud through various collaborative approaches. One method is self-regulation, where companies voluntarily adopt rules, such as codes of conduct or corporate social-responsibility initiatives, without direct government involvement. While this can improve transparency and consumer trust, it may lack strong enforcement. A more structured approach is co-regulation, which combines industry-led efforts with government oversight, often through public–private partnerships and data sharing (see, for example, Case study 5 in this publication). Initiatives such as the GFSI support this model by offering guidance on food-fraud prevention within food-safety systems. In addition, cooperation between governments, businesses, consumers and international organizations can lead to impactful joint actions. An example is Operation OPSON, a global campaign led by Europol and INTERPOL to combat counterfeit and substandard food. Another tool is the use of transnational contracts in global supply chains, which can include technical standards, quality requirements and third-party certifications to prevent fraud. However, these contracts can be difficult to monitor, may lack accountability to consumers, and can be undermined by actors who do not follow the rules. Despite these challenges, the private sector remains a key partner in building more transparent and trustworthy seafood supply chains.

Some important fish-importing markets have established instruments to fight food fraud in the food sector in general and in the aquatic sector in particular. For example, the European Union has established Regulation (EC) No 178/2002 of the European Parliament and of the Council, which outlines the general principles and requirements of food law, emphasizing the importance of food safety. Specifically, Article 8 on the protection of consumer interests states that (European Union, 2002):

> Food law shall aim at the protection of the interests of consumers and shall provide a basis for consumers to make informed choices in relation to the foods they consume. It shall aim at the prevention of:
>
> a) fraudulent or deceptive practices;
> b) the adulteration of food; and
> c) any other practices which may mislead the consumer.

Another legislative instrument that is applicable to fraud is Regulation (EU) 2017/625, which aims to guarantee that legal practices strengthen official control checks and, as such, may help fight fraudulent and deceptive practices. Regulation (EU) No 1169/2011 on Food Information to Consumers (FIC) and Regulation (EU) No 1379/2013 on the common organisation of the markets in fishery and aquaculture products represent the detailed legislation on identification and labelling of fishery and aquaculture products.

In the Unites States of America, federal legislation plays an important role in reducing the incidence of seafood mislabelling and fraud, which may also support more sustainable fisheries and strengthen seafood supply-chain resilience. The US FWS Lacey Act of 1900 (US 16 U.S.C. §§ 3371-3378) and the US Magnuson-Stevens Fishery Conservation and Management Act (16 U.S.C. 1801-1882, 90 Stat. 331 § 2) are

long-standing acts that prohibit the selling of fish in violation of state, federal, or foreign laws. The Seafood Import Monitoring Program (NOAA 15 CFR part 902, Vol 81) is a mandatory federal reporting procedure for imported fish and fish products from initial harvest to arrival at the US port of entry, particularly species at high risk of IUU fishing or seafood fraud. The Country of Origin Labelling regulation (USDA 7 CRF Part 60) requires importers and vendors to retain seafood origin and production data from the US port of entry to the end consumer. Logistically, the National Oceanic and Atmospheric Administration's (NOAA's) National Seafood Inspection Laboratory (60 Stat. 1087, U.S.C. 1621) supports US seafood commerce and trade by providing analytical services for seafood safety and quality, and the NOAA Seafood Inspection Program (7 U.S.C. 1621) provides voluntary seafood-product inspections, grading, process audit and export certification, among others.

CHAPTER 7

Analytical tools to detect food fraud in the aquatic sector

7.1 METHODS FOR FISH-SPECIES IDENTIFICATION (OR VERIFICATION) AND DIFFERENTIATION (OR DISCRIMINATION)

The available methods for identifying fish species can be divided into three broad groups: protein-based methods, DNA-based methods and chemical-fingerprinting methods. Protein-based methods include established techniques focusing on the analysis of physico-chemical differences, amino-acid composition and antigenic properties of muscle-tissue proteins consisting of myofibril proteins (myosin, actin, tropomyosin and troponin), connective and stromal proteins (elastin and collagen), and sarcoplasmic proteins (enzymes involved in the intermediary metabolism of the muscle cells) (Lago *et al.*, 2014). Despite the view that most of the traditional and official methods used in species identification are based on the analysis of protein markers (Rasmussen and Morrissey, 2008; Teletchea, 2009; Lago *et al.*, 2014), DNA-based methods appear to be by far the most widely used (Griffiths *et al.*, 2014; Naaum and Hanner, 2016).

The use of DNA-based methods presents a number of advantages over protein-based methods. First, phylogenetic studies have shown that DNA harbours a greater amount of information than proteins due to the degeneracy of the genetic code and the presence of many non-coding regions (Teletchea *et al.*, 2005). Whereas proteins vary with tissue type, age and status, DNA is largely independent of these factors. In fact, DNA is present in all cell types and therefore it can be extracted from any tissue, allowing the collection and storage of samples at any stage of the fishery chain. An additional key feature is that, although DNA can be altered by various types of processing, such as canning and heating, it is generally more resistant and thermostable than proteins (Teletchea *et al.*, 2005). In fact, protein-based methods can only be used to analyse fresh samples, as proteins in processed products denature upon heating. In contrast, short DNA fragments can usually be recovered and used for seafood identification even in highly processed products (Teletchea *et al.*, 2005; Naaum and Hanner, 2016). Overall, methods based on DNA analysis are more effective because of higher specificity and sensitivity, and because DNA can be amplified from few molecules even in degraded samples (Böhme *et al.*, 2019). Therefore, DNA-based methods, which include a wide range of techniques and equipment, have become increasingly relevant for addressing food-authentication problems. Most of the methods currently in use rely on polymerase chain reactions (PCRs) for the survey of informative DNA present in food matrices (Böhme *et al.*, 2019). The resulting PCR amplicons are then analysed (differently – depending on the method) to reveal the characteristic polymorphisms under study. The cytochrome c oxidase subunit I (COI), the cytochrome b (cytb) and the 16S ribosomal RNA (16S rRNA) mitochondrial genes have been the most targeted for fish-species identification. DNA barcoding, proposed by Hebert *et al.* in 2003, has been claimed to be the most efficient method for identifying living beings, and has also become a key player in ensuring the high quality of foodstuffs (Galimberti *et al.*, 2015, 2019). In 2011, the United States Food and Drug Administration proposed it as official method for seafood-species identification (Handy *et al.*, 2011). Teletchea (2009) reported that three methods were used particularly for fish-species identification, namely restriction fragment length polymorphism (RFLP), PCR sequencing and species-specific PCR.

More recently, in a survey conducted across European Union private and official laboratories, Griffiths *et al.* (2014), confirmed that DNA-based methods were far more prevalent, although the use of protein-based methods, and especially isoelectric focusing (IEF), was still reported. Moreover, the authors showed that the most widely used DNA-based methods were forensically informative nucleotide sequencing (FINS) and RFLP. Recently, a systematic review and meta-analysis performed by Luque and Donlan (2019) to characterize seafood mislabelling worldwide highlighted that most of the available studies on the topic (94 percent) relied on DNA-based methods, while protein-based methods, such as IEF and immunological assays, were used far less. Finally, species identification using next-generation sequencing (NGS) has gained considerable popularity in recent years, and it is expected that this innovative DNA-based technology will be increasingly used in the future (Lo and Shaw, 2018). In DNA-based methods, both nuclear and mitochondrial-specific genes are used as molecular targets. The following sections discuss traditional and innovative protein- and DNA-based methods.

7.1.1 Protein-based methods

7.1.1.1 Electrophoretic techniques: isoelectric focusing and two-dimensional electrophoresis

Electrophoresis is a technique applied to separate charged molecules through a solution or a gel by means of an electric field. The mobility and the separation of the molecules depend on the net charge of the molecule, the size and shape of the molecule, the ionic strength, the field strength, and the properties of the matrix through which the molecule migrates. Proteins are amphoteric compounds whose net charge depends on the pH-dependent ionization of amino acid side-chain carboxyl and amino groups. Thus, each protein species is characterized by a pH value, called the isoelectric point, or pI, at which the molecule has no net charge as a result of obtaining an equal number of negative and positive charges. Therefore, the pH-dependent mobility of proteins can be used to separate them by their isoelectric points through the application of an electrophoretic technique called IEF (Verrez-Bagnis *et al.*, 2019). The use of the IEF technique with water-soluble sarcoplasmic proteins has been successfully applied for species authentication in white fish species (*Flatfish* and *Sparidae*), catfish, tilapia, snapper, tuna, bonito, mackerel, swordfish (*Xiphias gladius*) and spearfish, puffer fish and shrimp (Rehbein *et al.*, 1995; Bossier and Cooreman, 2000; Renon *et al.*, 2005; Ataman *et al.*, 2006; Ortea *et al.*, 2010). The method has been validated by the United States Food and Drug Administration (FDA) since the 1990s and was established by the Association of Official Analytical Collaboration as the official method of fish-species identification in 1995 (AOAC Official Method 980.16: "Identification of Fish Species - Thin Layer Polyacrylamide Gel Isoelectric Focusing Method"). It is one of the official methods applicable for seafood regulatory control (https://www.fda.gov/food/science-research-food/regulatory-fish-encyclopedia-rfe). However, the technique is not suitable for the identification of species in cooked and smoked products because the heat treatments induce pronounced denaturation and degradation of the proteins, resulting in the loss of characteristic protein bands in the IEF (Verrez-Bagnis *et al.*, 2019). In order to expand the use of electrophoretic methods for the acquisition of species-identification patterns, alternative protocols based on urea IEF or sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) have been proposed and applied to seafood-species identification in processed products (Etienne *et al.*, 2000; Mackie *et al.*, 2000). Further evolution of electrophoretic techniques was represented by the combination of the SDS-PAGE technique and IEF through the implementation of two-dimensional electrophoresis (2-DE) protocols. In 2-DE, proteins are sequentially

separated according to pI and molecular weight, allowing the differentiation of species that share an identical IEF profile. Profiles of sarcoplasmic proteins obtained through 2-DE have been successfully used to differentiate several fish categories and to identify species in both fresh and heat-treated seafood (Valenzuela *et al.*, 1999; Piñeiro *et al.*, 2001; Chen *et al.*, 2004; Berrini *et al.*, 2006). In addition, 2-D electrophoretic analysis is the preliminary step in selecting proteins and peptides for identification analysis using spectrophotometric techniques, described in Section 7.6.

7.1.1.2 Immunological and chromatographic methods: enzyme-linked immunosorbent assay and high-performance liquid chromatography

Alternative analytical approaches to electrophoretic techniques include the use of immunoassays, specifically enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC) (Civera, 2003; Teletchea, 2009; Lago *et al.*, 2014). Immunological methods are based on specific antigen-antibody (Ag-Ab) binding reactions. Muscle proteins have been applied in several studies as antigens for the production of polyclonal or monoclonal antibodies in the setting of ELISA tests and strip immunoassay to identify several fish and seafood species (An *et al.*, 1990; Huang *et al.*, 1995; Céspedes *et al.*, 1999; Asensio *et al.*, 2003, Asensio *et al.*, 2008; Gajewski *et al.*, 2009). Two major limitations in the identification of standardizable ELISA methods are the difficulty in selecting antibodies with a high degree of specificity and the incidence of cross-reactions with non-target species, and the thermostability of antigenic determinants, which limits the applicability of such methods to the analysis of processed products (Ruethers *et al.*, 2020). High-performance liquid chromatography is an analytical method that separates molecules using their polarity according to their distribution between a polar mobile phase and an organic phase that is fixed to a matrix. The use of HPLC, in addition to making it possible to obtain species-specific protein chromatographic profiles, enables the quantitative estimation of the profiles returned by the analysis, making the method also applicable to the analysis of composite products and mixtures of fish products (Lago *et al.*, 2014). High-performance liquid chromatography protocols have been successfully applied for fish identification since the 1990s (Armstrong, 1992; Asensio *et al.*, 2001). Nevertheless, according to Lago *et al.*, (2014), the main HPLC disadvantage is represented by protein degradation occurring during the preparation of the samples prior to the chromatographic test. Indeed, according to the authors, this process can generate sarcoplasmic-protein clumping, potentially distorting the final chromatogram resulting from the HPLC analysis.

7.1.1.3 Spectrometric methods

Spectrometric methods applied to species identification and specifically mass spectrometry (MS) (mainly matrix-assisted laser desorption/ionization-time of flight [MALDI-TOF] and electrospray-ion trap mass spectrometry) fall under the umbrella of proteomic techniques and are recognized as reliable and accurate tools for food-product authentication (Carrera *et al.*, 2013). These methods cannot be considered stand-alone techniques and must be coupled with preliminary chromatographic or electrophoretic methods for the selection of peptide markers for identification analysis. Therefore, for seafood authentication purposes, they are generally associated with preliminary selection protocols in 2-DE electrophoresis or HPLC (Rodríguez and Ortea, 2017; Verrez-Bagnis *et al.*, 2019; Zambonin, 2021). In particular, at the European level, a MALDI–TOF MS–based method has been validated for unambiguous identification of different relevant species belonging to the Gadiform, Perciform and Pleuronectiform orders (Mazzeo and Siciliano, 2016; Stahl and Schröder, 2017).

An obstacle to the widespread use of the technique for monitoring the fish supply chain is the need to implement reference databases to identify the protein fingerprints obtained from the unknown samples for the selected peptide biomarkers (Stahl and Schröder, 2017).

7.1.2 Traditional DNA-based methods

7.1.2.1 Methods based on DNA sequencing

Introduction to Sanger sequencing (first generation sequencing)

The DNA sequencing method developed by Sanger *et al.* (1977) forms the basis of automated "cycle" sequencing reactions. This type of sequencing, also known as the chain-termination method, works as a classical PCR on the purified PCR amplicons using the same primers jointly with a DNA polymerase and a mix of deoxynucleotide triphosphate (dNTPs) and dideoxynucleotides (ddNTPs). These ddNTPs lack a 3′-OH group that is required for the formation of a phosphodiester bond between two nucleotides. Therefore, their incorporation stops the extension of the DNA strand. The DNA sample is divided into four separate sequencing reactions, containing all four of the standard dNTPs (dATP, dGTP, dCTP and dTTP), the DNA polymerase, and only one of the four ddNTPs (ddATP, ddGTP, ddCTP, or ddTTP) for each reaction. The ddNTPs are fluorescently labeled for detection in automated sequencing machines. The four reactions can be incorporated into a single reaction run and the DNA sequence can be read from fluorescent labels. A camera captures the fluorescence emission spectra. Each of the four ddNTPs has its own unique fluorescent spectrum; thus there are four possible fluorescent emission spectra. The emissions captured by the camera are converted to a readable form called an electropherogram, from which the sequence of the DNA of interest can then be determined. Thus, the electropherogram (also known as a trace) is a graphical representation of data received from a sequencing machine (Soper *et al.*, 2003)

Forensically informative nucleotide sequencing

Forensically informative nucleotide sequencing (FINS) is a method that combines DNA sequencing and phylogenetic analysis. It is used to identify samples based on informative nucleotide sequences. The sequence with the lowest genetic distance, or number of nucleotide substitutions, from the target amplicon represents the species group to which the original sample belongs (Bartlett and Davidson, 1992). Since FINS is based on nucleotide-sequence substitutions, it is important to select a fragment that exhibits high interspecies variability but low intraspecies variability in order to avoid ambiguities in the determination of species (Rasmussen and Morrissey, 2008). FINS represents a reliable chance to assess the nature of seafood products and to verify the information reported on the label. To date, numerous studies selecting different genetic markers have applied this technique to fish-species identification (Blanco *et al.*, 2008; Espiñeira *et al.*, 2009; Vinas and Tudela, 2009; Lago *et al.*, 2011; Armani *et al.*, 2012; Lago *et al.*, 2012; Chen *et al.*, 2012; Huang *et al.*, 2014; Santaclara *et al.*, 2014; Armani *et al.*, 2015a; Espiñeira and Vieites, 2015; Velasco *et al.*, 2016; Galal-Khallaf, *et al.*, 2017; Acutis *et al.*, 2019; Sivaraman *et al.*, 2019; Kim and Kang, 2023), to cephalopods (Chapela *et al.*, 2002; Santaclara *et al.*, 2007, Wen *et al.*, 2017) and to other unconventional seafood products (Wen *et al.*, 2010; Armani *et al.*, 2013).

DNA barcoding

In this method, after DNA extraction, specific DNA regions (barcodes) are amplified by PCR (using universal primers), sequenced and compared with a database of reference sequences (Hellberg and Morrissey, 2011). Originally, Hebert *et al.* (2003)

proposed a ~650-bp portion of the COI gene as a barcode for all living organisms. Despite some recognized limitations (Moritz and Cicero, 2004; Rennstam Rubbmark *et al.*, 2018; Tinacci *et al.*, 2018a), the COI barcode has so far succeeded in providing species-level resolution across diverse groups of insects, birds, fishes and primates and has also demonstrated the ability to differentiate species in other compartments of life, including protists and fungi (Hanner and Gregory, 2007).

DNA barcoding has been especially used for fish and seafood authentication (Handy *et al.*, 2011; Hellberg and Morrissey, 2011; Nicolè *et al.*, 2012; Wallace *et al.*, 2012; Nehal *et al.*, 2021; Fernandes *et al.*, 2021; Giusti *et al.*, 2023a). In some cases, the processing and preservation methods used with seafood products are not conducive to DNA barcoding with the full-length target-gene region. Thus, it is often appropriate resort to the use of a shorter region, known as "mini DNA barcodes" (Meusnier *et al.*, 2008; Hajibabaei and McKenna, 2012; Horreo *et al.*, 2013; Sarri *et al.*, 2014; Shokralla *et al.*, 2015; Armani *et al.*, 2015b; Mitchell and Hellberg, 2016; Günther *et al.*, 2017). Most seafood authentication studies have relied on the DNA database GenBank as a source of sequence information. GenBank is an expansive collection of all publicly available DNA sequences for genes in a multitude of species. It is produced by the National Center for Biotechnology Information and can be accessed at their website (http://www.ncbi.nlm.nih.gov). Moreover, the Barcode of Life Data Systems - BOLD (http://www.boldsystems.org/) has gained worldwide popularity with the development and success of DNA barcoding, based on the use of the COI gene as a target region for species identification and discrimination (Hebert *et al.*, 2003; Ratnasingham and Hebert, 2007). Although these databases are freely accessible and provide sequence information for many species, they have been criticized for their susceptibility to misidentification of species or population, missing information and inconsistent terminology (Rasmussen and Morrissey, 2008; Giusti *et al.*, 2019; Verrez-Bagnis *et al.*, 2019). Since the taxonomic accuracy within publicly available genetic databases represents a key factor for the reliability of the results, targeted preliminary analysis concerning the database reliability or the ex-novo building of in-house reference databases is recommended (Giusti *et al.*, 2019).

7.1.2.2 Methods not requiring DNA sequencing

Singleplex and multiplex PCR

Singleplex PCR is used to detect a single-target sequence of DNA thanks to the use of species-specific primers designed on single-base polymorphisms. Such primers generate a fragment, visualized by agarose gel electrophoresis, only in the presence of DNA from a given species. Multiplex PCR is a variant of singleplex PCR and permits the simultaneous amplification of many targets in the same reaction. Due to its rapidity and simplicity of execution, it is considered an alternative method particularly apt for screening purposes to minimize expenses and save time (Armani *et al.*, 2014). In this method, DNA from target species is amplified using a combination of species-specific primers, resulting in amplicon lengths that vary with species. A given species can be identified by the appearance of an amplicon of appropriate size throughout the electrophoretic run. The main challenge in setting up an efficient multiplex PCR is designing the primer. As mentioned previously, primers should be characterized by a good level of specificity. The number of regions of a certain gene that differ sufficiently among all the species to be able to distinguish between them is, however, limited. Consequently, the possibility of alternatives is also limited. As such, the higher the number of species, the lower the potential number of these alternatives. Therefore, the number of species included in the assay undoubtedly influences its effectiveness. This is also the case because the technique is based on a delicate equilibrium among

the species-specific primers, and the presence of many pairs in the reaction increases the chance of obtaining unspecific primer annealing on the sample DNA as well as spurious amplification products and may increase the possibility of the formation of primer dimers (Giusti *et al.*, 2016). Therefore, primers with a low capability of heterodimerization should be chosen. If this is not possible, the number of primers in the reaction mix should be reduced. One option is to choose a common forward or reverse primer. Moreover, it is recommended that primers with very similar optimum annealing temperatures be used (Castigliego *et al.*, 2015). Another fundamental step for obtaining a specific amplification, strictly connected with primer concentration, is the selection of an adequate DNA-template concentration. In fact, if the primer-to-template ratio is too low, specific products will not accumulate exponentially, while primer dimers may be amplified more efficiently than the desired target. Additionally, Taq polymerase, dNTPs and MgCl2 concentration should be appropriately evaluated, as well as the cycling condition (Giusti *et al.*, 2016). Appropriate controls should also be included to preclude the possibility of false positive or negative results. (The lack of amplified fragment on the gel may be due to technical problems rather than to the absence of the target DNA [Teletchea, 2009]). Several studies applying a multiplex PCR assay for seafood detection have been reported. Among the main seafood targets are groupers (Trotta *et al.*, 2005), tunas and mackerels (Lin and Hwang, 2008; Catanese *et al.*, 2010; Kim *et al.*, 2021; Lee *et al.*, 2022), cods and haddocks, anglerfish (Castigliego *et al.*, 2015), salmon and trouts (Rassmussen *et al.*, 2010), small pelagics (Armani *et al.*, 2012), gemfish (*Rexea solandri*) (Giusti *et al.*, 2016), pufferfish (Sangthong *et al.*, 2014; Dong *et al.*, 2019; Nan *et al.*, 2021), sciaenids (Barbosa *et al.*, 2020) and sharks (Cardeñosa *et al.*, 2017; 2018). In addition, bivalves (Marín *et al.*, 2013), gastropods (Chan *et al.*, 2012), cephalopods (Lee *et al.*, 2022), crustaceans (Suwannarat *et al.*, 2017) and jellyfish (Armani *et al.*, 2014) have also been included in studies applying a multiplex PCR assay.

Real-time PCR and high-resolution melting analysis

Real-time PCR (also known as quantitative PCR, real-time quantitative PCR, or qPCR) is a method of simultaneous DNA amplification and detection (Teletchea, 2009). It is an automated process, where no post-PCR processing is required to analyse the amplification output. In this way, the chances of post-PCR contact contamination decrease, as it is possible to observe and analyse RT-PCR products without removing them from the instrument. This is attributable to the technique's ability to detect, at every cycle of the PCR, the amount of PCR product (amplicon) using fluorescence (Salihah *et al.*, 2016). A fluorescent reporter molecule is included in the assay mix and monitored with an optical thermocycler that provides fluorescent excitation and quantification of the fluorescent emission. The fluorophores may be covalently linked to an oligonucleotide to form a labelled primer or probe or may be free molecules that bind to double-stranded DNA. Many different designs are possible, the common feature being that they must exhibit a change in fluorescence during PCR so that product accumulation can be monitored. An RT-PCR read-out is given as the number of PCR cycles ("cycle threshold" Ct) necessary to achieve a given level of fluorescence. The most popular real-time PCR assay, known as the TaqMan approach, is based on the hybridization of a dual-labelled probe to the PCR product and the development of a signal by loss of fluorescence quenching as PCR degrades the probe (Ponchel *et al.*, 2003). This approach has been used to authenticate several seafood species: cod and hake (Taylor *et al.*, 2002), tuna and mackerel (Lopez and Pardo, 2005; Prado *et al.*, 2013; Velasco *et al.*, 2013), flatfish (Herrero *et al.*, 2012), grouper (Chen *et al.*, 2018), cyprinids (Bajzik *et al.*, 2012), smelt (Baerwald *et al.*, 2011), puffer fish (Luekasemsuk *et al.*, 2015) and ling (*Molva molva*) (Taboada *et al.*, 2017), as well as cephalopods (Velasco *et al.*, 2020) and bivalves (Klapper and Schröder, 2021). Another common

approach is based on the binding of the fluorescent dye SYBR-Green I into the PCR product (PE Applied Biosystems, Warrington, UK) (Castigliego *et al.*, 2015; Chuang *et al.*, 2012). High-resolution melt analysis (HRMA) is the quantitative analysis of the melt curves of product DNA fragments following PCR amplification. High-resolution melt analysis requires a real-time PCR-detection system with excellent thermal stability and sensitivity, and HRMA-dedicated software. The combination of improved quantitative PCR instrumentation and saturating DNA-binding dyes has permitted the identification of small variations in nucleic-acid sequences by the controlled melting of double-stranded PCR amplicons (Garritano *et al.*, 2009). HRMA has been used to authenticate cods (Fernandes *et al.*, 2017; Tomßs *et al.*, 2017; Shi *et al.*, 2020), cyprinids (Behrens-Chapuis *et al.*, 2018), sharks (Cardeñosa *et al.*, 2017), eels (Noh *et al.*, 2018), salmon and trout (Xu *et al.*, 2021), and catfish (Buddhachat *et al.*, 2021), as well as bivalves (Jin *et al.*, 2015; Jilberto *et al.*, 2017; Del Rio-Lavín *et al.*, 2021) and crustaceans (Fernandes *et al.*, 2017b; Mondal and Mandal, 2020; Sharma *et al.*, 2020).

Restriction fragment length polymorphism

Restriction fragment length polymorphism, or RFLP, is a commonly employed tool to check the small but specific variations in a sequence of double-stranded DNA. It is based on the specificity of restriction endonucleases, which recognize a set of nucleotides called a restriction site and cleave the DNA at those sites. A specific RFLP pattern emerges during the electrophoretic separation of digested DNA, producing variable lengths of cleavage fragments that are characteristic of a sequence of DNA. Most of the studies applying this technique to fish and seafood authentication were published around one or two decades ago (Wolf *et al.*, 1999, 2000; Cocolin *et al.*, 2000; Hold *et al.*, 2001; Quinteiro *et al.*, 2001; Sanjuan *et al.*, 2002; Comesana *et al.*, 2003; Aranishi *et al.*, 2005; Khamnamtong *et al.*, 2005; Akasaki *et al.*, 2006; Hsieh *et al.*, 2007; Di Finizio *et al.*, 2007; Santaclara *et al.*, 2007; Espiñeira *et al.*, 2008; Pascoal *et al.*, 2008; Rea *et al.*, 2009; Hsieh *et al.*, 2010; Wen *et al.*, 2010; Fernández-Tajes *et al.*, 2011; Armani *et al.*, 2012; Chen *et al.*, 2012; Pascoal *et al.*, 2012; Chen *et al.*, 2014; Chairi and Rebordinos, 2014; Pappalardo and Ferrito, 2015; Sumathi *et al.*, 2015; Ferrito *et al.*, 2016). In the more recent studies, RFLP has been applied to the authentication of snappers (Sivaraman *et al.*, 2018), groupers (Anjali *et al.*, 2019) and tunas (Mata *et al.*, 2020; Yao *et al.*, 2020), as well as bivalves (Razak *et al.*, 2019; Giusti *et al.*, 2022) and other invertebrates (Zeng *et al.*, 2018). The use of RFLP has lessened recently in favour of other techniques.

Microarrays

PCR products can be analysed by hybridization to species-specific oligonucleotide probes arrayed on DNA microarrays, which can contain from several thousand to millions of DNA probes attached like small spots on the array surface. Upon hybridization of labelled PCR products, species can be identified directly, based on the pattern of positive probes. Seafood authentication using DNA microarrays is still a niche application (Verrez-Bagnis *et al.*, 2019), and only a few studies targeting marine species have been published so far (Kochzius *et al.*, 2010; Handy *et al.*, 2014; Kappel *et al.*, 2020).

Isothermal amplification

Loop-mediated isothermal amplification (LAMP) is a sensitive strand-displacement technique (Notomi, 2000). This method amplifies target DNA from a few to 109 copies in less than an hour under isothermal conditions. It is an offshoot of basic strand-displacement techniques, which have been described thoroughly (Notomi, 2000). Briefly, four highly specific primers are constructed from the target

DNA. One set of primers anneals to the target region – one after the other, on the same strand, and the primer that anneals at the later stage displaces the strand formed by the first primer with the help of Bst DNA polymerase. The Bst polymerase has a strand-displacement activity. This takes place on both strands, and the primers are designed such that loops are formed. The reaction is carried out under isothermal conditions as denaturation of the strand takes place by strand displacement. The reactions produce a series of stem-loop DNAs of various lengths. The four primers hybridize against six distinct sequences in the target DNA, making it highly specific (Savan *et al.*, 2005). Colorimetric LAMP, typically relying on the naked-eye evaluation of colour change, which is achieved through the use of different indicators such as pH, metal binding or DNA binding dyes, is the most popular LAMP application (Papadakis *et al.*, 2022). The use of LAMP has taken hold in recent years for the authentication of cod (Wang *et al.*, 2019; Hanyue *et al.*, 2023), tuna and tuna-like fish (Xiong *et al.*, 2021; Ali *et al.*, 2022; Xu *et al.*, 2022), salmon and trout (Xiong *et al.*, 2020; Xiong *et al.*, 2021; Li *et al.*, 2022) and flatfish (Deconinck *et al.*, 2023; Wax *et al.*, 2023), as well as cephalopods (Ye *et al.*, 2017) and crustaceans (Benjakul and Saetang, 2022).

7.1.3. Innovative DNA-based methods

7.1.3.1 Methods based on high-throughput sequencing

Introduction to Next Generation Sequencing (NGS) Technologies

Next-generation sequencing (NGS) technologies are high-throughput methods able to simultaneously sequence all the DNA molecules, including those present in trace amounts (Goodwin *et al.*, 2016). Unlike Sanger sequencing, where a single amplicon from a single species is amplified and a unique sequence is obtained, in this case, hypothetically, 100 percent of the DNA contained in a sample can be amplified and sequenced each time (Morey *et al.*, 2013). NGS technologies are grouped into second (2nd GS), third (3rd GS) and fourth generation (4th GS) sequencing, though there is no consensus on this classification (Fernandes *et al.*, 2021). The 2nd GS technologies rely on the cyclic parallel reading of clonally amplified and spatially separated amplicons (Mardis, 2008). A number of 2nd GS technology platforms were developed. Illumina is the current market leader. Currently, it has four benchtop sequencers (iSeq, MiniSeq, MiSeq and NextSeq) and two production-scale platforms (HiSeq and NovaSeq). Other well-known 2nd GS platforms include Ion Torrent by Thermo Fisher Scientific, Pyrosequencing by Roche 454 and SOLiD by Life Technologies. One of the main weaknesses that have been recognized in 2nd GS technologies is that the maximum read length that can be obtained is not as long as that of Sanger sequencing (Morey *et al.*, 2013). With respect to the 2nd GS, the Illumina platform produces a maximum of 2 × 300 bases for paired-end reads, available only on the MiSeq sequencer or, at production-scale level, on the Novaseq sequencer (Haynes *et al.*, 2019). Ion Torrent reached a maximum read length of 600 bp with Ion GeneStudio S5 sequencers. The 3rd GS and 4th GS technologies have gone beyond this limit, and are able to routinely generate reads in excess of 10 kb (Pollard *et al.*, 2018). The 3rd GS commercially available platforms are Helicos BioSciences and Pacific BioSciences, while the 4th GS is uniquely represented by Oxford Nanopore Technologies, where single molecules of DNA can be identified by passing them through a tiny channel with the potential to produce very long reads (Deamer *et al.*, 2016; Pervez *et al.*, 2022). The first commercially available instrument, Oxford Nanopore MinION technology, produced by Oxford Nanopore Technologies, heralds the promise of a USB-sized, portable DNA sequencer (Pollard *et al.*, 2018). However, a major drawback is the high raw-read error, which can range from 10 percent to 22 percent (Baloğlu *et al.*, 2021). Indeed, nanopore sequencing is still limited by low single-passage de novo sequencing accuracy,

compared with that of other established sequencing platforms (Ku and Roukos, 2013; Noakes *et al.*, 2019). Different from other fields of investigation, such as the analysis of biodiversity in environmental samples or trophic interactions, where this technique is widely used (Ruppert *et al.*, 2019), its application in foodstuffs is still limited, mainly due to a factual lack of method standardization (Haynes *et al.*, 2019; Giusti *et al.*, 2023b; Giusti *et al.*, 2024;). Two main applications are available for NGS technologies: metabarcoding and shotgun sequencing. Basically, they are both articulated in: 1) library preparation, 2) sequencing and 3) final data analysis using bioinformatic pipelines (Staats *et al.*, 2016). A pipeline is generated using a collection of software and algorithms with the aim of producing an accurate features table with potential taxa contained in a sample (Hakimzadeh *et al.*, 2023).

Metabarcoding

The combination of NGS with DNA barcoding has been termed metabarcoding. It is a method of targeted NGS that analyses genetic variation in specific genomic regions. The method uses PCR to create sequences of DNA called amplicons. Multiplexing – barcoding samples so that they can be mixed into pools – allows multiple samples to be sequenced on a single sequencing run. Before multiplexing, individual samples used for amplicon sequencing must be transformed into libraries by adding adapters and enriching target regions by PCR amplification. The adapters allow the formation of indexed amplicons and enable the amplicons to adhere to the sequencing flow cell. To date, metabarcoding has been applied to the authentication of canned tuna (Kappel *et al.*, 2020); salmon (Wang *et al.*, 2021); complex seafood products such as fish burgers, cakes and surimi (Carvalho *et al.*, 2017; Giusti *et al.*, 2017; Piredda *et al.*, 2022; Mottola *et al.*, 2022; Giusti *et al.*, 2023b); and sea cucumber (Wang *et al.*, 2021). Recently, a systematic review was published on the application of metabarcoding to the authentication of food of animal origin (Giusti *et al.*, 2024).

Shotgun sequencing

This method involves randomly breaking up the genome into small DNA fragments that are sequenced individually. A computer program looks for overlaps in the DNA sequences, using them to reassemble the fragments in their correct order to reconstitute the genome. Metabarcoding has the potential to determine the presence of different species in a mixture, but this approach often falls short in estimating the correct relative abundance of individual species in the mixture (Hellberg *et al.*, 2017; Lo and Shaw, 2018; Shokralla *et al.*, 2015; Xing *et al.*, 2019). In fact, the PCR step in the barcoding approach is prone to bias due to its dependency on degenerate primers, which assume equal amplification of the target gene from all species. Furthermore, the common use of mitochondrial target genes, such as COI, though it increases the sensitivity, also increases the possibility of bias due to fluctuating levels of mitochondrial DNA per cell, tissue or age. Thus, using shotgun sequencing and avoiding the PCR step altogether would be beneficial for accurately quantifying the biological content of mixed-food products. Approaches using shotgun-metagenome sequencing have successfully quantified the content of mixed-food products, demonstrating the potential for this technique in food and feed control (Haiminen *et al.*, 2019; Kobus *et al.*, 2020). However, this approach is currently poorly applied (Varunjikar *et al.*, 2022).

Table 1 provides a comparison of various protein-based, traditional DNA-based and innovative DNA-based analytical methods for identifying and differentiating fish species.

TABLE 1
Analytical methods for fish-species identification and differentiation

Category of analytical methods	Analytical technique group (specific analytical technique)	Benefits	Limitations	Sources
Protein-based methods	Electrophoretic (Isoelectric focusing [IEF])	Simple, accurate and inexpensive IEF results in the proteins being confined to small zones, leading to enhanced resolution and sensitivity	Not suitable for heat-treated foodstuffs	Tokur and Korkmaz, 2023 Verrez-Bagnis *et al.*, 2017
Protein-based methods	Electrophoretic (2-dimensional electrophoresis [2-DE])	Can be successfully applied to fresh and heat-treated foodstuffs	Resulting protein profiles include several bands, making their interpretation sometimes challenging Requires skilled operators and appropriate equipment	Tokur and Korkmaz (2023) Tokur and Korkmaz(2023)
Protein-based methods	Immunological (Enzyme-linked immunosorbent assay [ELISA])	Useful in heat-sterilized products	Difficulty in selecting antibodies with a high degree of specificity and incidence of cross-reactions with non-target species, and the thermostability of antigenic determinants, which limits the applicability of such methods to the analysis of processed products	Civera *et al.*, 1996 Lago *et al.*, 2014; Teletchea *et al.*, 2005 Ruethers *et al.*, 2020
Protein-based methods	Chromatographic (High-performance liquid chromatography [HPLC])	Allows the obtention of species-specific protein chromatographic profiles, the quantitative estimation of the profiles returned by the analysis, making the method also applicable to the analysis of composite products and mixed-seafood products	Protein degradation occurring during sample preparation preliminary to the chromatographic test	Lago *et al.*, 2014 Fiorino *et al.*, 2018
Protein-based methods	Spectrometric (Matrix-assisted laser desorption/ionization-time of flight [MALDI-TOF] mass spectrometry)	Generally associated with preliminary selection protocols in 2-DE electrophoresis or HPLC	Not a stand-alone technique; must be coupled with preliminary chromatographic or electrophoretic methods for selection of peptide markers for identification analysis	Rodríguez and Ortea, 2017 Verrez-Bagnis *et al.*, 2017 Zambonin, 2021 Carrera *et al.*, 2013
Protein-based methods	Spectrometric (Electrospray-ion trap [ESI-IT] mass spectrometry)		Not a stand-alone technique; must be coupled with preliminary chromatographic or electrophoretic methods for selection of peptide markers for identification analysis	Carrera *et al.*, 2013 Rodríguez and Ortea, 2017 Verrez-Bagnis *et al.*, 2017 Zambonin, 2021
Traditional DNA-based methods	Based on DNA sequence (Sanger sequencing [first-generation sequencing])	Fast and cost effective – the gold standard for accurate detection of single nucleotide	Limited throughput; may not detect mosaicism. Can require larger amount of input DNA than massively parallel sequencing	Sanger *et al.*, 1977 Bhérer *et al.*, 2024
Traditional DNA-based methods	Based on DNA sequence (Forensically informative nucleotide sequencing [FINS])	Accurate method of species identification of a specimen when this is not possible by conventional means		Rasmussen and Morrissey, 2008 Bartlett, 1992
Traditional DNA-based methods	Based on DNA sequence (DNA barcoding)	Testable and reproducible system as a link is maintained between any barcode and a voucher specimen In most cases, faster and cheaper than traditional morphological identifications for massive routine identifications	Potential false negatives can occur, i.e., different DNA barcodes between individuals belonging to the same species due to ancestral polymorphism or genetic introgression	Hebert *et al.*, 2003 Ratnasingham and Hebert, 2007

TABLE 1
Analytical methods for fish-species identification and differentiation (continued)

Category of analytical methods	Analytical technique group (specific analytical technique)	Benefits	Limitations	Sources
Traditional DNA-based methods	Do not require DNA sequence (Singleplex PCR)	Due to its rapidity and simplicity of execution, considered an alternative method, particularly for screening purposes to minimize expenses and save time	Challenge in designing the primer to set up efficient multiplex PCR Primers should have a good level of specificity. There are a limited number of regions of a certain gene that differ sufficiently among all the species for purposes of distinguishing between them. Thus, alternatives are limited, and the higher the number of species, the lower the potential number of alternatives.	Armani *et al.*, 2014 Giusti *et al.*, 2016
Traditional DNA-based methods	Don't require DNA sequence (Multiplex PCR)	Variant of Singleplex PCR permitting simultaneous amplification of many targets in the same reaction Rapidity and simplicity of execution make it an option particularly for screening purposes to minimize expenses and save time.	Challenge in designing the primer to set up efficient multiplex PCR. Primers should have a good level of specificity. There are a limited number of regions of a certain gene that differ sufficiently among all the species for the purpose of distinguishing between them. Alternatives are also limited – the higher the number of species, the lower the potential number of alternatives.	Armani *et al.*, 2014 Giusti *et al.*, 2016
Traditional DNA-based methods	Don't require DNA sequence (Real-time PCR [qPCR])	Provide fast and high-throughput detection and quantification of target DNA sequences in different matrices	May not be effective for detecting unknown or highly variable sequences without prior knowledge of the target DNA	Salihah *et al.*, 2016 Teletchea, 2009
Traditional DNA-based methods	Don't require DNA sequence (High-resolution melting analysis)	Fast, simple procedure with high reproducibility and capability of analysing multiple CpG sites within a region	Different heterozygotes may produce melting curves so similar to that, although they clearly vary from homozygous variants, they are not differentiated from each other.	Hattori and Ushijima, 2027 Wittwer, 2009
Traditional DNA-based methods	Don't require DNA sequence (Restriction fragment length polymorphism [RFLP])	Fast, simple, accurate molecular tool for profiling and identifying population	Has lower discriminatory power and more expensive to run compared to RAPD	Martya *et al.*, 2012 Smith et al., 2002
Traditional DNA-based methods	Don't require DNA sequence [Isothermal Amplification (LAMP])	Significant advantage: it can be conducted at a stable temperature (e.g. in dry block heater or incubator) Products can be detected much faster than in standard techniques, sometimes only requiring analysis with the naked eye.	Sensitive to cross-contamination Difficult to check samples for the presence of reaction inhibitors, as requires two reactions – one to detect inhibitors and another to amplify the material	Soroka and Rymaszewska, 2021
Innovative DNA-based methods	Based on high-throughput sequencing (Next-generation sequencing [NGS] technologies)	High-throughput, able to simultaneously sequence all the DNA molecules, including those present in trace amounts	Application in foodstuffs still limited, mainly due to factual lack of method standardization	Giusti *et al.*, 2024 Haynes *et al.*, 2019
Innovative DNA-based methods	Based on high-throughput sequencing (Metabarcoding)	Allows multiple samples to be sequenced on a single sequencing run	Can determine the presence of different species in a mixture but often falls short in estimating correct relative abundance of individual species in the mixture	Hellberg *et al.*, 2017 Lo and Shaw, 2018 Xing *et al.*, 2019
Innovative DNA-based methods	Based on high-throughput sequencing (Shotgun sequencing)	Can quantify content of mixed-food products, thus has potential for use in food and feed control		Haiminen *et al.*, 2019 Kobus *et al.*, 2020

Note: All the methods detect these types of food fraud: counterfeit and simulation, species substitution and mislabelling.

7.2 METHODS FOR DIFFERENTIATION BETWEEN FRESH AND FROZEN-THAWED FISH

Selling frozen/thawed fish as fresh fish is one of the forms of adulteration in the fisheries and aquaculture sector. Fresh fish is defined as an unprocessed fish product, whether whole or gutted, that has not undergone any treatment to ensure preservation, other than chilling (for instance, in the definition of "fresh fishery products" in the European Regulation (EC) No 853/2004 Art. 2 Annex I No 3.5) (EU, 2004). Fish is a perishable food with continuous changes at the molecular level and in chemical composition, due to relatively rapid postmortem processes that depend on storage conditions. Therefore, the freshness of fish is considered an important aspect of its quality (Rimbach *et al.*, 2015). Freezing, salting, drying, smoking, soaking in acids or edible oil have all been used as preservation methods for fish (Ebermann and Elmadfa, 2008). Freezing best preserves the original consistency and properties of the fish flesh (Rimbach *et al.*, 2015; Qiao *et al.*, 2022), enabling extensive international trade (Tülsner, 1996). Furthermore, complex supply chains increase the risk of food fraud (Ellefson *et al*, 2013; Hong *et al.*, 2017), as fresh fish is traded at a higher price compared to frozen-thawed fish (Hassoun *et al.*, 2020a; Verrez-Bagnis *et al.*, 2018). The correct labelling of frozen-thawed fish is regulated by countries and/or associations of states. (For instance, in the European Union, Article 35 of regulation (EU) No 1379/2013 [EU, 2013] addresses this). Due to freezing, subsequent (deep-)frozen storage and thawing, ice-crystal formation and growth, recrystallization processes, melting and osmosis take place within fish tissue (Boziaris, 2014; Gökoğlu and Yerlikaya, 2015). This affects the consistency and chemical composition of the tissue: proteolysis (Belitz *et al.*, 2008; Gökoğlu and Yerlikaya, 2015; Sotelo *et al.*, 1995a), protein oxidation (Baron *et al.*, 2007; Nakazawa and Okazaki, 2020; Sotelo *et al.*, 1995b), lipid oxidation (Baron *et al.*, 2007; Pirestani *et al.*, 2010), lipolysis (Baron *et al.*, 2007), texture changes (Belitz *et al.*, 2008; Nakazawa and Okazaki, 2020; Qiao *et al.*, 2022) and drip losses (Belitz *et al.*, 2008; Qiao *et al.*, 2022; Tülsner, 1996) have all been reported. In view of these postmortem changes, the differentiation between fresh and frozen-thawed fish is challenging. Currently, there are no standardized methods to investigate this type of fraud, and due to a lack of studies, there are no available statistics on the incidence of frozen-thawed fish sold as fresh fish. However, some analytical studies have been conducted to develop and investigate possible methods to differentiate between fresh fish and frozen-thawed fish, demonstrating the need for analytical tools for this kind of fraud in the fisheries and aquaculture sector. Table 2 summarizes the findings of these studies.

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish

Analytical method		Fish species	Aspects for further research	Reference
Category	Method specifics			
Enzymatic	α-glucosidase, β-N-acetyl-glucosaminidase	Cod (*Gadus morhua*), saithe (*Gadus virens*), red fish (*Sebastes marinus*), haddock (*Gadus aeglefinus*)	Influence of storage time (frozen fish)	Rehbein *et al.*, 1978
	α-glucosidase	Cod (*Gadus morhua*)	Comparison with other enzymes	Rehbein, 1979
	cytochrome oxidase	Rainbow trout (*Oncorhynchus mykiss*)	Additional determination of the protein content Influence of storage time (frozen fish)	Barbagli and Crescenzi, 1981
	β-N-acetylglucosaminidase	Carp (*Cyprznus carpio*), sea-bream (*Chrysophrys major*)	Influence of storage time (frozen fish)	Kitamikado *et al.*, 1990
	adenosine triphosphatase, lactate dehydrogenase	Mrigal (*Cirrhinus mrigala*), mullet (*Liza parsia*), pearlspot (*Etroplus suratensis*), milkfish (*Chanos chanos*), tilapia (*Oreochromis mossambicus*)	Accompanying determination of α-amino nitrogen, total volatile nitrogen, free fatty acids, thiobarbituric acid value	Nambudiri and Gopakumar, 1992
	B-hydroxyacyl-coenzyme A-dehydrogenase	Rainbow trout (*Oncorhynchus mykiss*)	Influence of storage time (fresh fish) Influence of storage temperature (fresh fish) Additional InvestigationInvestigation(*Procambarus clarkii*)	Hoz *et al.*, 1992
	A-glucosidase, β-N-glucosaminidase, acid phosphataseS	Rainbow trout (*Oncorhynchus mykiss*)	Additional determination of protein content Influence of storage time (fresh and frozen-thawed fish) Influence of freezing procedure Enzyme activity determination in press juice or in homogenized tissue	Nilsson and Ekstrand, 1993
	B-hydroxyacyl-coenzyme A-dehydrogenase	Mackerel (*Scomber scombrus*), tuna (*Thunnus alalonga*), sea-bream (*Pagellus centrodontus*), sole (*Solea solea*), hake (*Merluccius merluccius*)	Influence of storage time (fresh and frozen-thawed fish) Influence of freezing temperature Influence of fish size (hake)	Pavlov *et al.*, 1994

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish (continued)

Analytical method		Fish species	Aspects for further research	Reference
Category	**Method specifics**			
	B-hydroxyacyl-coenzyme A-dehydrogenase	Sole (*Solea solea*), sea-bream (*Pagellus centrodontus*), hake (*Merluccius merluccius*), gilt headed bream (*Sparus aurata*), seabass (*Dicentrarchus labrax*), salmon (*Salmo salar*)	Influence of freezing temperature Double-frozen fish	Fernández *et al.*, 1999
	α-glucosidase	Salmon (*Salmo salar*, *Oncorhynchus keta*)	Additional Investigation of smoked products	Rehbein and Çakli, 2000
	β-hydroxyacyl-coenzyme A-dehydrogenase, α-glucosidase	Plaice (*Pleuronectes platessa*), whiting (*Merlangus merlangus*), mackerel (*Scomber scombrus*)	Influence of storage time (fresh and frozen fish) Comparison with torrymeter measurements and a physiological method (eye lens)	Duflos *et al.*, 2002
	α-glucosidase, β-galactosidase, β-N-acetylglucosamidase	Anchovy (*Engraulis engrasicolus*), sardines (*Sardina pilchardus*), horse mackerel (*Trachurus trachurus*), chub mackerel (*Scomber japonicus colias*)	Additional sensory analysis of quality index, colour measurements, examination of the medulla of the crystalline lens Influence of storage time (frozen fish) Influence of fish species	Alberio *et al.*, 2014
	lactate dehydrogenase	Sea-bream (*Sparus aurata*)	Influence of storage time (fresh fish) Comparison with α-glucosidase	Diop *et al.*, 2016
	B-hydroxyacyl-coenzyme A-dehydrogenase	Yellowfin tuna (*Thunnus albacares*)	Influence of storage time (frozen fish)	Bernardi *et al.*, 2019
	α-glucosidase	Seabass (*Dicentrarchus labrax*)	Comparison with other methods (determination of protein content, free calcium concentration, nucleotides and related compounds concentration)	Marlard *et al.*, 2019
Morphological	Histological	Gilthead (*Sparus auratus*), red mullet (*Mullus barbatus*), swordfish (*Xiphias gladius*)		Bozzetta *et al.*, 2012
	Histological	European hake (*Merluccius merluccius*)		Tinacci *et al.*, 2018a
	Histological	Trout, among others	Examination of different meat samples	Orlova *et al.*, 2020
	Histological	Smoked salmon (*Salmo salar*)		Sigurgisladottir et al., 2000
	Histological	Smoked salmon (*Salmo salar*)		Pezzolato *et al.*, 2020
	Histological	Black rockfish (*Sebastes melanops*), steelhead trout (*Oncorhynchus mykiss*)		Kagan and Viner, 2022
	Histological	Marinated anchovy fillets (*Engraulis encrasicolus*)		Meistro *et al.*, 2016
	Physiological (eye lens, transparent or opaque)	Plaice (*Pleuronectes platessa*), whiting (*Merlangus merlangus*), mackerel (*Scomber scombrus*)	Only applicable for whiting and mackerel, not for plaice Influence of storage time (fresh and frozen fish) Comparison with enzymatic examinations and torrymeter measurements	Duflos *et al.*, 2002

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish (continued)

Analytical method		Fish species	Aspects for further research	Reference
Category	**Method specifics**			
Spectroscopy	Raman spectroscopy, multivariate data analysis (PCA)	Horse mackerel (*Trachurus trachurus*), European anchovy (*Engraulis encrasicolus*), red mullet (*Mullus surmuletus*), bluefish (*Pomatamus saltatrix*), salmon (*Salmo salar*), flying gurnard (*Trigla lucerna*)	Double-frozen fish	Velioğlu *et al.*, 2015 Zhu *et al.*, 2013
	NIR, multivariate data analysis (PCA, multiple linear regression [MLR])	Horse mackerel (*Trachurus trachurus*)		Uddin and Okazaki, 2004
	NIR, multivariate data analysis (PCA, PLS-DA, OPLS-DA)	Sea-bream (*Sparus aurata*), red mullet (*Mullus barbatus*), sole (*Solea vulgaris*), swordfish (*Xiphias gladius*)		Ottavian *et al.*, 2013
	NIR, MIR, multivariate data analysis (PCA, LDA, SIMCA)	Atlantic mullet *(Pseudupeneus prayensis)*		Alamprese and Casiraghi, 2015
	NIR, multivariate data analysis (PCA, PLS, LDA, logistic regression [LR], RF, extreme gradient boosting [XGB], SVM)	Alaskan pollock (*Gadus chalcogrammu*), cod (*Gadus morhua*), European plaice (*Pleuronectes platessa*), sole (*Solea solea*), turbot (*Psetta maxima*)	Influence of the position of the measurement	Gonçalves *et al.*, 2021
	NIR, multivariate data analysis (PLS-DA)	Bigeye tuna (*Thunnus obesus*)	Influence of the addition of water and additives to the tissue Comparison with bioelectrical impedance analysis and time domain reflectometry	Nieto-Ortega *et al.*, 2022
	NIR, multivariate data analysis (PLS-DA)	Mackerel (*Scomber scombrus*)	Influence of freezing procedure Double-frozen fish Comparison of two handheld NIR devices Influence of seasonal characteristics	Giró-Candanedo *et al.*, 2024
	NIR, multivariate data analysis (SIMCA, PLS-DA)	Carp (*Cyprinus carpio*)	Influence of measurement position Double-frozen fish	Atanassova *et al.*, 2024
	Vis/NIR, multivariate data analysis (SIMCA, PCA-DA)	Sea-bream (*Pagrus major*)		Uddin *et al.*, 2005
	Vis/NIR, multivariate data analysis (PCA, KNN, PLSR)	Cod (*Gadus morhua*)	Comparison of stationary instrument with handheld device	Sivertsen *et al.*, 2011

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish (continued)

Analytical method		Fish species	Aspects for further research	Reference
Category	**Method specifics**			
			Influence of measurement position	
			Influence of storage time (fresh fish)	
	Vis/NIR, multivariate data analysis (PLS-DA, descriptive principal-component scores)	Swordfish (*Xiphias gladius*)	Influence of storage temperature (frozen fish)	Fasolato *et al.*, 2012
	Vis/NIR, multivariate data analysis (PCA, KNN, PLSR)	Salmon (*Salmo salar*)	Influence of measurement position	Kimiya *et al.*, 2013
	Vis/NIR, multivariate data analysis (PCA, LS-SVM)	Halibut (*Psetta maxima*)	Influence of freezing procedure	Zhu *et al.*, 2013
	Vis/NIR, multivariate data analysis (PCA, PLS-DA)	West African goatfish (*Pseudupeneus prayensis*)		Ottavian *et al.*, 2014
	Vis/NIR, multivariate data analysis (SIMCA, LS-SVM, probabilistic neural network [PNN])	Grass carp (*Ctenopharyngodon idellus*)	Influence of storage temperature (frozen fish)	Cheng *et al.*, 2015
	Vis/NIR, multivariate data analysis (PCA, PLS-DA)	Tuna (*Thunnus thynnus*)	Influence of blooming (myoglobin oxygenation) Influence of water content and measurement temperature on the measurements	Reis *et al.*, 2017
	Vis/NIR, multivariate data analysis (PCA, KNN)	Cod (*Gadus morhua*)	Double-frozen fish Influence of freezing procedure Influence of thawing procedure Influence of storage time (frozen fish)	Washburn *et al.*, 2017
	Vis/NIR, multivariate data analysis (principal-component analysis [PCA] and sequential feature selection [SFS], several machine-learning classifiers)	Red snapper (*Lutjanus campechanus*)	Double-frozen fish	Qin *et al.*, 2020
	Vis/NIR, multivariate data analysis (PLS-DA)	Pearl gentian grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*)	Influence of measurement position Influence of storage times (fresh and frozen-thawed fish)	Chen *et al.*, 2021
	MIR, multivariate data analysis (PCA, FDA)	Whiting (*Merlangius merlangus*)	Influence of freezing procedure Influence of thawing procedure	Karoui *et al.*, 2007
	MIR, multivariate data analysis (PCA, FDA)	Sevruga (*Acipenser stellatus*)	Influence of storage time (fresh fish prior freezing)	Vilkova *et al.*, 2023

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish (continued)

Analytical method		Fish species	Aspects for further research	Reference
Category	**Method specifics**			
			Accompanying electrophoresis analyses	
	Fluorescence, multivariate data analysis (PCA, FDA)	Whiting (*Merlangius merlangus*)	Influence of freezing procedure	Karoui *et al.*, 2006
			Influence of thawing procedure	
	Fluorescence, multivariate data analysis (PCA, FDA)	Seabass (*Dicentrarchus labrax*)	Additional determination of pH, moisture content, texture-profile analysis, color measurements	Karoui *et al.*, 2017
			Influence of storage time (fresh fish, fresh fish prior freezing, frozen-thawed fish)	
	1H NMR, quantitative, multivariate data analysis (PCA)	Salmon (*Salmo salar*)	Concentration of aspartate as indicator	Shumilina *et al.*, 2020
	1H NMR, multivariate data analysis (PCA-LDA)	Mackerel (*Scomber scombrus*), trout (*Oncorhynchus mykiss*, *Oncorhynchus aguabonita*, *Salmo trutta fario*), cod (*Gadus morhua*)	Influence of freezing procedure	Kaltenbach *et al.*, 2024
			Investigation of polar and lipid metabolites	
			Investigation via 13C NMR, 31P NMR	
	MRI	Rainbow trout (*Oncorhynchus mykiss*)	Influence of freezing procedure	Nott *et al.*, 1999a
			Double-frozen fish	
			Influence of storage time (frozen fish)	
	MRI	Cod (*Gadus morhua*), mackerel (*Scomber scombrus*)	Influence of storage time (frozen fish)	Nott *et al.* 1999b

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish (continued)

Analytical method		Fish species	Aspects for further research	Reference
Category	**Method specifics**			
Mass spectrometry	SPME-GC/MS	Seabream (*Sparus aurata*)	Accompanying determination of peroxide value, thiobarbituric acid-reactive substances index Influence of storage time (frozen fish) Identification of metabolite markers (1-octen-3-ol, 1-penten-3-ol and Z-4-heptenal)	Iglesias *et al.*, 2009
	SPME-GC/MS, multivariate data analysis (PCA, ascending hierarchical classification [AHC])	Seabass (*Dicentrarchus labrax*), sea-bream (*Sparus aurata*), cod (*Gadus morhua*), salmon (*Salmo salar*)	Accompanying microbiological Investigations (total aerobic flora, *Pseudomonas spp.*, *Shewanella putrefaciens*) and total volatile basic nitrogen/trimethylamine assay Influence of storage time (frozen fish) Identification of metabolite markers (dimethyl sulfide, 3-methylbutanal, ethyl acetate and 2-methylbutanal)	Leduc *et al.*, 2012
	HPLC-HRMS, multivariate data analysis (PCA)	Salmon (*Salmo salar*), bullet tuna (*Auxis rochei*)	Influence of freezing procedure (for salmon only) Influence of storage time (frozen fish) Identification of metabolite markers (arginine and its metabolites for salmon, phosphated choline/ etanolamine derivates for bullet tuna)	Chiesa *et al.*, 2020
	DART-HRMS, multivariate data analysis (PLS-DA, SVM)	Seabass (*Dicentrarchus labrax*)	Univariate analysis of the most important 25 m/z values Application on salmon (Salmo salar)	Massaro *et al.*, 2021
	LC-HRMS multivariate data analysis (PCA, PLS-DA)	Seabass (*Dicentrarchus labrax*)	Identification of metabolite markers (eicosapentaenoic acid, docosahexaenoic acid) Application on salmon (Salmo salar)	Stella *et al.*, 2022

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish (continued)

Analytical method		Fish species	Aspects for further research	Reference
Category	**Method specifics**			
Electrical	Torrymeter	Plaice (*Pleuronectes platessa*), whiting (*Merlangus merlangus*), mackerel (*Scomber scombrus*)	Influence of storage time (fresh and frozen fish) Comparison with enzymatic examination and a physiological method (eye lens)	Duflos *et al.*, 2002
	Impedance	Grass carp (*Ctenopharyngodon idellus*), tilapia (*Oreochromis niloticus*)	Influence of storage time of fresh and frozen-thawed fish	Zhang *et al.*, 2010
	Impedance, multivariate data analysis (PCA, DA)	Salmon (*Salmo salar*)	Additional texture measurement, ATP-related compounds (k-value), moisture, pH, total volatile basic nitrogen, thiobarbituric acid, water-holding capacity, drip loss, microbiological Investigations (mesophilic counts, *Enterobacteriaceae*) Influence of storage time (frozen fish) Double-frozen fish	Fernández-Segovia *et al.*, 2012
	Impedance, multivariate data analysis (PCA, DA)	Sea-bream (*Sparus aurata*)	Additional texture measurement, ATP-related compounds (k-value), moisture, pH, total volatile basic nitrogen, thiobarbituric acid, water-holding capacity, drip loss, microbiological Investigations (mesophilic counts, *Enterobacteriaceae*) Influence of storage time (frozen fish) Double-frozen fish	Fuentes *et al.* 2013
	Impedance, multivariate data analysis (PCA, LDA, SVM, back propagation artificial neural network [BPANN], PLS)	Salmon (*Salmo salar*)	Additional total volatile basic nitrogen determination Influence of storage time (fresh fish)	Sun *et al.*, 2020
	Bioelectrical impedance analysis, multivariate data analysis (PLS-DA)	Bigeye tuna (*Thunnus obesus*)	Influence of the addition of water and additives to the tissue Comparison with NIR and time-domain reflectometry	Nieto-Ortega *et al*., 2022
	Bioimpedance, multivariate data analysis (SVM, LDA, KNN, RF)	Salmon (*Salmo salar*)	Multiple frozen fish	D. Zhang *et al.*, 2024a
	Time-domain reflectometry, multivariate data analysis (PLS-DA)	Bigeye tuna (*Thunnus obesus*)	Influence of the addition of water and additives to the tissue Comparison with bioelectrical impedance analysis and NIR	Nieto-Ortega *et al*., 2022

TABLE 2
Overview of published analytical methods to differentiate between fresh and frozen-thawed fish (continued)

Analytical method		Fish species	Aspects for further research	Reference
Category	**Method specifics**			
Electrophoresis	Two-dimensional gel electrophoresis	Seabass (*Dicentrarchus labrax*)	Accompanying analyses of biogenic amines and total volatile basic nitrogen Influence of storage time (fresh fish) Identification of markers (MS/MS, parvalbumins)	Ethuin *et al.*, 2015
Mitochondrial permeability measurement		Sea-bream (*Sparus aurata*)	Comparison of two different measurement methods Influence of storage time (fresh fish)	Bouchendhomme *et al.*, 2022
Muscle-fibre permeability measurement		Sea-bream (*Sparus aurata*)	Comparison of two different measurement methods Influence of storage time (fresh fish)	Bouchendhomme *et al.*, 2023
Free calcium concentration		Seabass (*Dicentrarchus labrax*)	Comparison with other methods (activity of α-glucosidase, determination of protein content and concentration of, nucleotides and related compounds)	Marlard *et al.*, 2019
Nucleotides and related compounds		Seabass (*Dicentrarchus labrax*)	Comparison with other methods (activity of α-glucosidase, determination of protein content, free calcium concentration)	Marlard *et al.*, 2019

Notes: DA: discriminant analysis; DART-HRMS: direct analysis in real-time high-resolution mass spectrometry; FDA: factorial discriminant analysis; HPLC-HRMS: high-performance liquid chromatography coupled with high-resolution mass spectrometry; KNN: k-nearest neighbour classifier; LC-HRMS: liquid chromatography coupled to high-resolution mass spectrometry; LDA: linear discriminant analysis; LS-SVM: least squares support vector machine; MIR: mid-infrared; MRI: magnetic resonance imaging; MS/MS: tandem mass spectrometry; NIR: near-infrared; NMR: nuclear magnetic resonance; OPLS-DA: orthogonal partial least squares discriminant analysis; PCA: principal-components analysis; PCA-LDA: principal-components analysis in combination with linear discriminant analysis; PLS: partial least squares; PLS-DA: partial least squares discriminant analysis; PLSR: partial least squares regression; RF: random forest; SIMCA: soft independent modeling of class analogy; SPME-GC/MS: solid phase microextraction/gas chromatography/mass spectrometry; SVM: support vector machine; vis: visible.

Hassoun *et al*. (2020a) summarized studies focusing on methods used to differentiate between fresh and frozen-thawed fish and other seafood. The promising methods described in their summary, along with those described in more recently published studies, are presented in Table 2. Besides the literature listed in Table 2, other methods that were tested showed no success in terms of differentiating fresh and frozen-thawed fish. For example, regarding drip loss, no significant differences were observed in the water content of fresh, frozen-thawed and double-frozen-thawed rainbow trout (*Oncorhynchus mykiss*) samples (Popelka *et al*., 2014). Negligible differences in shelf life and bacterial spoilage between fresh and frozen-thawed fish have been reported (Fagan *et al*., 2003; Popelka *et al*., 2014; Yin *et al*., 2014). However, process parameters in the technological implementation can influence shelf life and microbial growth of frozen-thawed fish (Boziaris, 2014; Popelka *et al*., 2014).

Regarding the techniques listed in Table 2, it should be noted that the methods differ in their applicability. For example, some methods are suitable for fish fillets, parts of fish fillets and for entire fish as the sample (for instance, nuclear magnetic resonance [NMR] analysis [Kaltenbach *et al*., 2024]), while other methods can only be conducted on whole-fish samples (this includes physiological examination of the eye lens [Duflos *et al*., 2002]). Some approaches are non-destructive (such as direct near-infrared (NIR) measurement on the fillet [Kimiya *et al*., 2013), while others are destructive (for instance, in the required sample preparation (Massaro *et al*., 2021]). Some methods require negligible or no sample preparation (such as NIR analysis [Karoui *et al*., 2006]), while others rely on extensive sample preparation (as in the case of fat extraction prior to Raman analysis [Velioğlu *et al*., 2015]). Lastly, handheld devices, such as NIR, TDR and bioelectrical impedance analysis [Nieto-Ortega *et al*., 2022]) allow for portable quality control, while other methods (including mass spectrometry [Massaro *et al*., 2021]) require expensive, stationary equipment.

Most methods are based on enzymatical, morphological (in particular, histological) or spectroscopic investigations, in addition to other approaches (including mass spectrometry, impedance, nucleotides and related compounds). Freezing and thawing significantly increases the activity of various mitochondrial enzymes (such as β-hydroxyacyl-coenzyme A-dehydrogenase [Bernardi *et al*., 2019; Duflos *et al*., 2002; Fernández *et al*., 1999; Pavlov *et al*., 1994]) and lysosomal enzymes (such as α-glucosidase [Alberio *et al*., 2014; Duflos *et al*., 2002; Marlard *et al*., 2019; Nilsson and Ekstrand, 1993; Rehbein *et al*., 1978; Rehbein, 1979; Rehbein and Çakli, 2000]), as well as enzymes from blood cells (including β-N-acetylglucosaminidase [Alberio *et al*., 2014; Kitamikado *et al*., 1990; Nilsson and Ekstrand, 1993; Rehbein *et al*., 1978]). Therefore, it is hypothesized that these enzymes are released from the cell organelles because of the freezing and thawing procedure (Bernardi *et al*., 2019; Hassoun *et al*., 2020a; Kitamikado *et al*., 1990; Rehbein and Çakli, 2000). Some authors suggest that the analysis of the activity of lysosomal enzymes is more specific than the analysis of the activity of mitochondrial enzymes and is therefore a preferred approach (Rehbein *et al*., 1978). One limitation of enzymatic methods, however, is that they are fish-species specific (Verrez-Bagnis *et al*., 2018).

Morphological examinations are based on changes in the fish flesh structure or the tissue due to the freezing and thawing procedure. These changes include freeze/thaw artifacts in the flesh (histological examination, such as in Bozzetta *et al*. [2012]) or appearance of the eye lens (transparent or opaque, physiological examination, such as in Duflos *et al*. [2002]). However, for a histological differentiation, it is already known that freeze/thaw artifacts in the flesh of the fish depend on the freezing rate and therefore on the freezing procedure (Hassoun *et al*., 2020a). Furthermore, these methods are time consuming and require highly experienced assessors (Verrez-Bagnis *et al*., 2018).

More recently, the topic has been investigated using comprehensive spectroscopic techniques (especially NIR and Vis/NIR) and multivariate data analysis. Moreover, mass spectrometry was used as an analytical technique. These methods depend on an authentic data basis to predict unknown samples as "fresh" or "frozen-thawed". Specific techniques permit the identification of possible marker substances to detect fresh or frozen-thawed fish. For example, Shumilina *et al.* (2020) – in a storage trial, analysis of trichloroacetic acid extracts of the fish flesh using NMR - suggested aspartic acid as a marker substance for frozen-thawed salmon, while in a Chiesa *et al.* (2020) storage trial, analysis of perchloric acid extracts using non-targeted HPLC-HRMS proposed arginine and its derivatives as suitable markers for frozen-thawed salmon. Aspartic acid was not mentioned as a relevant metabolite in the study by Chiesa *et al.* (2020), and arginine was not mentioned as a relevant metabolite to distinguish fresh from thawed salmon in the study by Shumilina *et al.* (2020). As reported in another study, dimethylamine was suggested as a marker for frozen-thawed (or otherwise processed) cod (Martinez *et al.*, 2005). However, Howell *et al.* (1996) refuted a generally valid dimethylamine formation in frozen fish. Further research is needed to confirm reliable marker substances.

Additionally, many studies demonstrate that some influences on the differentiation exist and more are conceivable. Due to extensive postmortem processes, the storage time of fresh, frozen-thawed samples should be considered (Chaijan *et al.*, 2006; Chiesa *et al.*, 2020; Ciampa *et al.*, 2012; Rehbein and Oehlenschläger, 2009; Shumilina *et al.*, 2015; Shumilina *et al.*, 2020; Tenyang *et al.*, 2017), as should the storage time of frozen fish (Baron *et al.*, 2007; Pirestani *et al.*, 2010; Sánchez-Alonso *et al.*, 2012; Sánchez-Alonso *et al.*, 2014; Suárez-Medina *et al.*, 2024) and the storage temperature of frozen fish (Baron *et al.*, 2007; Fasolato *et al.*, 2012; Howell *et al.*, 1996; Sánchez-Alonso *et al.*, 2014; Sotelo *et al.*, 1995a). Furthermore, the freezing procedure (Sánchez-Alonso *et al.*, 2014; Vidaček *et al.*, 2008; J. Zhang *et al.*, 2024b) and the thawing procedure (Gökoğlu and Yerlikaya, 2015; Javadian *et al.*, 2013), as well as multiple freezing and thawing cycles, also have an impact on the fish sample (Benjakul and Bauer, 2001; Giró-Candanedo *et al.*, 2024; Pinto *et al.*, 2020; Samantaray *et al.*, 2021; Strateva *et al.*, 2021; Velioğlu *et al.*, 2015; Vidaček *et al.*, 2008; Washburn *et al.*, 2017). Additional treatments to conserve the quality of stored fish, such as superchilling (European Commission Delegated Regulation EU 2022/2258, [EU, 2022]), controlled atmosphere (Gökoğlu and Yerlikaya, 2015; Kirtil and Oztop, 2016; Ruiz-Capillas and Moral, 2002; Sone *et al.*, 2012), and glazing (Boziaris, 2014; Evans, 2008) could be relevant. Especially for methods that rely on a database, the database used must be authentic and of appropriate size while comprising the required variations (for example, fish species, production method [aquaculture, wild catch], producer, diet, seasonal fluctuations, storage time, storage temperature, freezing and thawing procedure). Consequently, differences in fresh and frozen-thawed fish could depend on several factors. Some studies contain investigations of relevant aspects for which further research is needed (see the column "Further investigations or considered aspects" in Table 2).

Applying a general method to differentiate between fresh fish and frozen-thawed fish is questionable. Studies of methods used to differentiate between fresh and frozen-thawed fish show advantages and disadvantages of the different methods available. In addition, analytical methods (for instance, for official food-control systems or industry-based quality-control systems) should ideally be fast, non-destructive, and should not require reagents or expensive equipment, therefore, the differentiation of fresh and frozen-thawed fish hides analytical challenges. To establish reliable methods, the robustness of the method should be verified against as many influences as possible. Further research and standardization of methods is needed, also to enable an assessment of the occurrence of frozen-thawed fish sold as fresh fish in the fisheries and aquaculture sector.

7.3 METHODS FOR DIFFERENTIATION BETWEEN WILD-CAUGHT AND FARMED FISH

7.3.1 Genetic profiling

Seafood-fraud investigations often strive to identify individuals of the same species from wild and farmed sources. Landmark investigations by Karlsson *et al.* (2011) and Glover *et al.* (2011) have been conducted on key commercial species including Atlantic salmon and Atlantic cod (*Gadus morhua*), respectively. These investigations utilized genetic-profiling analyses to successfully discriminate between farmed and wild individuals regardless of their populations of origin. Karlsson *et al.* (2011) used 60 single-nucleotide polymorphisms (SNPs) to identify the source of individual salmon as either farmed or wild. The data from this study compared historical wild and farmed salmon populations based on 7 000 SNPs that were widely distributed on 27 of the 29 chromosomes (Karlsson *et al.*, 2011), suggesting that these techniques can be broadly used in other investigations using other, commercially relevant species. Glover *et al.* (2011) used ten microsatellite loci and the Pan I locus to screen samples of wild and farmed cod and showed that wild individuals were genetically distinct from farmed fish. Glover *et al.* (2011) concluded that these molecular genetic tools may be implemented to profile farmed and wild individuals of the same species and that other species could also be analysed to further support genetic introgression studies. Collectively, the approaches described here have important implications for seafood-fraud investigations involving the identification of farmed vs wild individuals (Glover *et al.*, 2011, Karlsson *et al.*, 2011).

7.3.2 Chemical profiling

Apart from genetic profiling, various chemical-profiling techniques have been used to verify claims about wild-caught fish and to discriminate them from aquaculture products.

Investigation of the fatty-acid composition can support the differentiation between farmed fish and wild fish. Analysis of the proximate and fatty-acid composition in the muscle of wild and farmed sardine (*Sardinella brasiliensis*) using gas chromatography coupled with flame ionization detection (GC-FID) indicated higher total lipid content in farmed fish, while the n-3 long chain and the n-6 polyunsaturated fatty acids (n-3 LC-PUFA and n-6 PUFA) were higher in wild and farmed sardine (Scheuer *et al.*, 2024). Busetto *et al.* (2008) examined the fatty-acid composition and isotopic signatures (carbon [$\delta^{13}C$] and nitrogen [$\delta^{15}N$]) using isotope ratio mass spectrometry (IRMS) to differentiate between wild-caught and farmed turbot (*Psetta maxima*). Additionally, 18:2-6 fatty acids and $\delta^{15}N$ were reliable determinants for classification purposes. Fiorino *et al.* (2019) employed the direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) technique, in combination with multivariate analysis, to analyse fish lipid extracts and achieved discrimination between wild and farmed salmon, studying the 30 most relevant DART-HRMS signals, which were assigned to fatty acids. Aursand *et al.* (2009) used ^{13}C-NMR untargeted profiling of muscle lipids and multivariate analysis to discriminate between wild and farmed Atlantic salmon. Probabilistic neural networks (PNN) and support vector machines (SVM) provided excellent discrimination scores (98.5 percent and 100.0 percent, respectively). Fourier transform infrared spectroscopy (FTIR) has also been used as an effective tool to discriminate between farmed and wild seabass based on lipid composition (Vidal *et al.*, 2014).

Isotopic fingerprinting through a compound-specific stable carbon isotope analysis approach, focussing on amino acid $\delta^{13}C$ profiling and multivariate analyses, allowed for accurate tracing of wild and farmed salmon, the latter from aquaculture in Norway

and Ireland. The $\delta^{13}C$ profiles of essential and non-essential amino acids demonstrated different potential regarding their source diagnostic abilities (Wang *et al.*, 2018).

The trace element profile in otoliths of fish, acquired by inductively coupled plasma-mass spectrometry (ICP-MS), combined with chemometrics, has been used to separate farmed fish from wild western Mediterranean Sea stocks of seabass (*Dicentrarchus labrax L.*) and sea-bream (*Sparus aurata L.*). Moreover, trace elements in otoliths allowed for the discrimination among wild fish stocks within each species (Arechavala-Lopez *et al.*, 2016).

The detection of the mislabelling of farmed salmonids as wild can be based on their carotenoid pigment profile. Astaxanthin is the natural carotenoid pigment in wild Atlantic salmon, while farmed fish contain canthaxanthin or synthetic astaxanthin, administered through the feed. This difference can be detected by thin-layer chromatography (Craik and Harvey, 1987) or high-performance liquid chromatography of a lipid extract (Megdal *et al.*, 2009; Ostermeyer and Schmidt, 2004).

Vibrational spectroscopy is gaining attention for its non-destructive and rapid profiling potential to discriminate between wild and farmed fish and other seafood. Rapid differentiation of wild and farmed European seabass (*Dicentrarchus labrax L.*) has been accomplished by near infrared spectroscopy coupled with chemometrics (Ghidini *et al.*, 2019a). Machine learning-assisted near- and mid-infrared spectroscopy has also been used for the rapid discrimination of wild and farmed Mediterranean mussels (*Mytilus galloprovincialis*). The best discrimination was observed using Fourier transform mid-infrared spectroscopy spectra of the interior part of mussels (Ayvaz *et al.*, 2024). Near-infrared reflectance spectroscopy (NIRS) and several machine-learning algorithms for both regression and classification tasks were explored by Gonçalves *et al.* (2021) to discriminate between farmed and wild samples of Alaskan pollock (*Gadus chalcogrammus*), Atlantic cod (*G. morhua*), European plaice (*Pleuronectes platessa*), common sole (*Solea solea*) and turbot (*Psetta maxima*), showing accurate classification of 88 percent.

7.4 METHODS FOR THE VERIFICATION OF THE GEOGRAPHICAL PROVENANCE OF FISH

In many countries, it is mandatory to declare the geographical origin of fishery products (EU, 2013). This applies to the fishing area for wild-caught fish and to the harvesting area and country of origin for aquaculture products. Particularly for wild-caught fish, illegal fishing in areas with catch restrictions can affect biodiversity and overall sustainability. Moreover, there are fishery products with a protected designation of origin (PDO) or protected geographical indication (PGI) (EU, 2025), for which there is also an additional interest in control from the producers' side. Finally, the geographical designation can be important for excluding an origin with a polluted environment or a region where ciguatera-producing algae occur (Mudge *et al.*, 2023).

7.4.1 Stable-isotope analysis

Stable-isotope ratio analyses of light mass elements (C, N, S, O, H) are reliable and proven methods for verifying the provenance of fish. Isotope ratios can be analysed by mass spectrometry in either bulk tissue or specific compounds. Different environmental conditions result in distinct stable isotopic signatures, primarily of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), being incorporated into biological tissues. During this process, the heavy isotopes of these elements become enriched in the food chain. Similarly, the other elements can provide valuable information about the fish's habitat and isotopic environment. For example, oxygen isotopes ($\delta^{18}O$) allow conclusions to be drawn about sea temperatures for marine fish, or the geographically varying signature of precipitation water in freshwater fish.

The combination of $\delta^{13}C$ and $\delta^{15}N$ in muscle tissue has been widely used to distinguish the geographical origin of various commercial fish species, such as mackerel, yellow croaker and pollock (Kim *et al*., 2015), as well as hake (Carrera and Gallardo, 2017), from different oceans. With the help of $\delta^{13}C$ and $\delta^{15}N$ data, isoscapes of pelagic fish and squid in the Northwest Pacific Ocean could also be derived, which show distinct spatial patterns across marine areas (Ohshimo *et al.*, 2019). In addition to $\delta^{13}C$ and $\delta^{15}N$, $\delta^{34}S$ has also been used to trace the geographical origin of Atlantic cod (Wilson *et al.*, 2024). Using linear discriminant analysis, some regions could be distinguished with strong predictive power (for instance, >90 percent), but an exact assignment of unknown samples across all examined marine areas was possible in only about 25 percent of cases.

The combination of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ in fillets was found to be effective in geographically differentiating Australian Murray cod from several Australian freshwater aquaculture sites (Turchini *et al*., 2009). The C and N isotopes reflected the feed, while the O isotopes reflected the culture water. In contrast to seawater, freshwater is strongly influenced by geography and its properties, such as the isotopic signature of precipitation and evaporation. It has been shown that the regional variation in freshwater isotope signatures can also be used to differentiate between fish from individual lakes and farms in Switzerland using $\delta^{18}O$ of their tissue water (Rossier *et al.*, 2014).

Although $\delta^{18}O$ in seawater shows only minor global variations of approximately 0.0 ± 1.0 ‰ in typical fishing areas, oxygen isotopes analysed in carbonate biominerals of marine animals ($\delta^{18}O_{biomin}$) exhibit larger differences. Isoscapes for $\delta^{18}O_{biomin}$ in fish, cephalopods and shellfish show global differences of approximately 0.0 ± 4.0 ‰ (Martino *et al.*, 2022), which are primarily driven by the strong reciprocal relationship between $\delta^{18}O_{biomin}$ and water temperature. Thus, analysing $\delta^{18}O_{biomin}$ in otoliths from fish allows for a rough estimate or the exclusion of a wider geographical origin.

In addition, otoliths in fish can be used to determine stable strontium isotopes ($\delta^{87}Sr$) by multicollector inductively-coupled plasma mass spectrometry (MC-ICP-MS), with these isotopes being determined by the geological characteristics of the catchment area, particularly in freshwater systems. This way, several studies to determine the natal origins of salmonid fish, such as Pacific salmon (Barnett-Johnson *et al.*, 2008), Chinook salmon (Brennan *et al.*, 2015) and Bering cisco (Padilla *et al*., 2015), were able to distinguish between different North American rivers using $\delta^{87}Sr$.

While the methods described so far involve analysing stable isotopes in bulk samples, it is also possible to conduct analyses at the individual molecular level by coupling isotope ratio mass spectrometry (IRMS) with gas chromatography. This compound-specific isotope analysis (CSIA) can be used, for example, to determine $\delta^{13}C$ in amino acids or monosaccharides. This made it possible to determine the geographical origin of sea cucumbers and Yesso scallops (*Patinopecten yessoensis*) (Zhao *et al.*, 2018, 2019).

Scientists using MC-ICP-MS instrumentation have created further novel techniques and applications regarding determination of the geographical origin of different species as well as source assignment modelling of ocean pollutants. The MC-ICP-MS system is used to conduct high-precision isotopic analysis of metals, metalloids and some non-metals. Ocean-science studies using species of commercial relevance have been conducted at small and large spatial scales to identify the geographical origin of individuals (Cransveld *et al*. 2017; Bank *et al*. 2024) and may be expanded to other species in support of seafood safety and provenance investigations. Cransveld *et al*. (2017), successfully discriminated European seabass (*Dicentrarchus labrax*) using mercury isotopic values of $\delta^{202}Hg$ and $\Delta^{199}Hg$, and bulk estimates of carbon and nitrogen isotopes, along with the SIBER package in program *R* to develop ellipses of

regional, isotopic niches. This approach successfully identified seven distinct seabass populations throughout Europe, highlighting the importance and potential of these methods to be applied at large spatial scales (for instance, throughout Europe). In a second case study, Bank *et al.* (2024) successfully used a multi-isotope composition approach. They analysed isotopes of lead, cadmium, copper, mercury, iron and zinc along with elemental composition to identify the geographical origin of brown crab (*Cancer pagurus*) at a small spatial scale using machine learning and Bayesian model-selection techniques. Both techniques hold strong potential for supporting seafood-fraud investigations where geographical origin and/or provenance needs to be assigned at small or large spatial scales.

7.4.2 Stable-isotope analysis combined with further techniques

To increase the accuracy of predictions regarding the geographical origin of seafood, it is best to combine isotope data with ancillary data from other analytical techniques. Data fusion enables a fingerprinting approach, which, combined with advanced statistical methods and machine-learning techniques, has considerable additional potential. The combination of isotope data and element profiles, which can comprise both bulk and trace elements, is widely used. Element profiles provide an accurate reflection of the geological environment, including aquatic transport of materials and the influence of food chains.

Element profiles can be analysed using either ICP-MS or x-ray fluorescence (XRF). The combination of $\delta^{13}C$ and $\delta^{15}N$ of mussel tissue and trace-elements contents of their shells has been shown to resolve the geographical origin of Mediterranean mussels within Europe, as well as those from Chile and Tunisia. Using a random forest model, only six variables ($\delta^{13}C$, $\delta^{15}N$, Pb, Ba, Mn, Al) were finally needed to correctly classify the mussels with an accuracy of 97 percent (del Rio-Lavin *et al.*, 2022). Similarly, the geographical differentiation of swimming crabs (*Portunus trituberculatus*) from three Chinese production areas was achieved by analysing $\delta^{13}C$, $\delta^{15}N$ and element profiles in various tissues (Xu *et al.*, 2022). Focusing on the profiling of lanthanide tracers in combination with $\delta^{13}C$ and $\delta^{15}N$ in the mantle tissue enabled the discrimination of different species and geographical origins of Mediterranean and Atlantic squid using classification and regression tree analysis (Varra *et al.*, 2024). Instead of ICP-MS, elemental profiling using XRF analysis, in combination with isotope analysis of $\delta^{13}C$ and $\delta^{15}N$, was applied in two Australian studies (Gopi *et al.*, 2019a, 2019b). Using discriminant analysis and random forest classification, the provenance of farmed and wild Asian seabass (*Lates calcarifer*) and that of black tiger prawns (*Penaeus monodon*) was determined with over 90 percent accuracy.

Data-fusion techniques involving three independent sources were used to trace the geographical origin of sea cucumbers in China (Kang *et al.*, 2021). This study chemometrically combined elemental dry matter concentrations of C, N, O and H, their respective isotope ratios and mineral element concentrations in body-wall tissue, achieving up to 100 percent accuracy. The provenance of blue mussels (*Mytilus edulis*) from China could be determined with 94 percent accuracy using explainable machine learning, by only combining isotopic and compositional data of C, N, O and H from mussel tissue (Kang *et al.*, 2023).

Another option for data fusion is the combination of stable-isotope and fatty-acid analyses. An exploratory study showed the potential of $\delta^{13}C$, $\delta^{15}N$ and fatty-acid profiles to trace the geographical origin of jumbo squid (*Dosidicus gigas*) using stepwise discriminant analysis (Gong *et al.*, 2018).

Table 3 provides an overview of different isotopic analytical techniques for verifying the provenance of fish.

TABLE 3
Isotopic analytical techniques for verifying the provenance of fish

Technique	Taxon	Matrix	References
IRMS (CN)	Fish, cephalopods	Defatted tissue	Kim *et al.*, 2015; Carrera and Gallardo, 2017; Ohshimo *et al.*, 2019
IRMS (CNS)	Fish	Dried tissue	Wilson *et al.*, 2024
IRMS (CNO)	Fish	Dried tissue	Turchini *et al.*, 2009
IRMS (O)	Fish	Tissue water	Rossier *et al.*, 2014
IRMS (O)	Fish, cephalopods, shellfish	Otoliths, statoliths, shells	Martino *et al.*, 2022
MC-ICP-MS (Sr)	Fish	Otoliths	Barnett-Johnson *et al.*, 2008; Brennan *et al.*, 2015; Padilla *et al.*, 2015
IRMS-CSIA (C)	Echinoderms, shellfish	Dried tissue	Zhao *et al.*, 2018; Zhao *et al.*, 2019
MC-ICP-MS (Hg)	Fish	Dried tissue	Cransveld *et al.*, 2017
MC-ICP-MS (multi-isotopic)	Crustacea	Dried tissue	Bank *et al.*, 2024
IRMS (CN) and ICP-MS (element fingerprint)	Shellfish, crustacea, cephalopods	Dried tissue and shells (first two studies), dried tissue (third study)	del Rio-Lavin *et al.*, 2022; Xu *et al.*, 2022; Varra *et al.*, 2024
IRMS (CN) and XRF (element fingerprint)	Fish, crustacea	Dried tissue	Gopi *et al.*, 2019a; Gopi *et al.*, 2019b
IRMS (CNHO) and ICP-MS (element fingerprint) and CHNO composition	Echinoderms	Dried tissue	Kang *et al.*, 2021
IRMS (CNHO) and CHNO composition	Shellfish	Dried tissue	Kang *et al.*, 2023
IRMS (CN) and fatty acids	Cephalopods	Defatted tissue/lipids	Gong *et al.*, 2018

Notes: IRMS: isotope ratio mass spectrometry; CN: carbon and nitrogen; CNS: carbon, nitrogen and sulphur; CNO: carbon, nitrogen and oxygen; O: oxygen; MC-ICP-MS: multi-collector inductively coupled plasma mass spectrometry; CSIA: compound-specific isotope analysis; ICP-MS: inductively coupled plasma mass spectrometry; XRF: X-ray fluorescence; CHNO: carbon, hydrogen, nitrogen and oxygen.

7.4.3 Non-isotope-based techniques

Chemical profiling by nuclear magnetic resonance (NMR) has been employed for seafood origin determination, often in conjunction with chemometric modelling. Heude *et al.* (2016) used ^{1}H-NMR spectroscopy and chemometrics to study the specific metabolic profile of aqueous extracts of caviar samples and differentiate Aquitaine caviar production from other productions, supporting the establishment of the protected geographical indication (PGI) for Aquitaine caviar. Kuhn *et al.* (2024) discriminated the origin of pikeperch (*Sander lucioperca*), European perch (*Perca fluviatilis*) and common bream (*Abramis brama*) from closely related water bodies (lakes and coastal sea regions), combining untargeted metabolomics by ^{1}H-NMR spectroscopy with statistical analysis and machine learning. The origin prediction was tolerant to seasonal variations. ^{13}C-NMR spectroscopy, along with the machine-learning tools probabilistic neural networks (PNN) and support vector machines (SVM) allowed for the differentiation of the geographical origin of Atlantic salmon from Canada, the Faroe Islands, Iceland, Ireland, Norway, Scotland and Tasmania, with correct classification rates ranging from 82.2 percent to 99.3 percent (Aursand *et al.*, 2009).

Rapid screening methods based on non-destructive, relatively low-cost and environmentally friendly analytical techniques have also been employed for investigating the geographical origin of fish and seafood. Recent studies have demonstrated the potential of benchtop and portable near-infrared spectroscopy equipment, in combination with chemometrics, to differentiate the geographical origin of oysters

(Guo *et al*., 2024), processed anchovies (Varrà *et al*., 2021), musky and common octopuses (Varrà *et al*., 2022), sea cucumber (*Apostichopus japonicus*) (Guo *et al*., 2018), European seabass (*Dicentrarchus labrax L.*) (Ghidini *et al*., 2019a) and tilapia fillet products (Liu *et al*., 2015).

7.5 METHODS FOR THE VERIFICATION OF ORGANIC AQUACULTURE PRODUCTION METHOD

Seafood consumers are often concerned about whether farmed fish is organic or not. Designation and certifications as organic vary by country, and definitions can also vary substantially, often driven by the composition and certification process of the fish feed and procedures used to grow the fish. Organic feed uses all-natural plant and animal ingredients and is produced without using synthetic substances such as pesticides, herbicides, antibiotics, or genetically modified organisms (GMOs). This is especially important as there has been a significant rise in organic aquaculture (Beg *et al*., 2024) and a growing interest in sustainable food choices. To date, research on this topic is lacking, although there are increases in attention toward these topics, as expressed by both scientists and policymakers (Perdikaris and Paschos 2010; Mente *et al*., 2011; Beg *et al*., 2024).

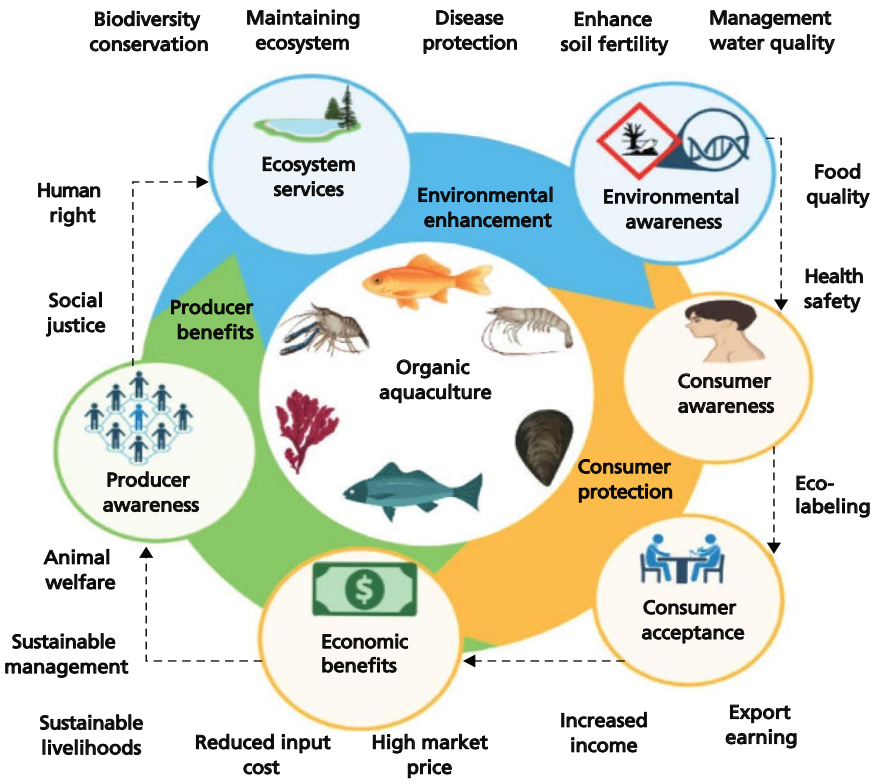
A literature review by Beg *et al*. (2024) summarized the prospects and challenges of organic aquaculture to meet sustainability goals. The review reported that consumers often lack a detailed understanding of the principles of organic food, and that regulations are especially difficult to apply consistently (Beg *et al*., 2024). Organically farmed fish is often perceived as healthier and of higher quality; and labelling conventionally raised fish as organic is considered a fraudulent practice. Differences in feed and nutrition are expected to result in differences in the quality of products of organic aquaculture, but this research area of seafood fraud is truly in its infancy and can be considered a significant knowledge gap, especially in terms of reliable analytical methods to differentiate organically raised from conventionally farmed fish. Future investigations should consider novel techniques and applications aimed at identifying organic seafood and may benefit from lessons learned from land-based food systems. Figure 1 illustrates the conceptual model and framework of organic aquaculture developed by Beg *et al.* (2024).

Stable-isotope and fatty-acid profile analyses have been employed to authenticate raw, smoked and graved organic salmonids. $\delta^{15}N$ and non-lipid $\delta^{13}C$ levels were significantly higher in organic salmon and trout than in conventionally farmed fish. Using $\delta^{15}N$ and lipid $\delta^{13}C$ even allowed the distinction of organic from wild salmon at the same time. Regarding fatty-acid profiles, the linoleic-acid content of organic salmon clearly ranged between wild and conventional levels, while organic trout was differentiated from conventional trout based on oleic and gondoic acid content (Molkentin *et al*., 2015). Compound-specific carbon stable isotope analysis and multivariate statistical processing of the amino acid $\delta^{13}C$ allowed for the discrimination between wild and organically raised salmon with high accuracy (Wang *et al*., 2018).

Stable-isotope analysis in several shrimp species allowed for the differentiation of organic and wild-caught animals using $\delta^{15}N$ and $\Delta\delta^{13}C$, where $\Delta\delta^{13}C$ is the difference in $\delta^{13}C$ between the lipid and non-lipid fraction. However, when using *Litopenaeus vannamei*, this combination successfully discriminated only between organically and conventionally farmed shrimps, but not between conventionally farmed and wild. Moreover, this species clearly showed higher content of the saturated fatty acids C15:0 and C17:0 in organic compared to conventionally farmed animals (Ostermeyer *et al.*, 2014).

FIGURE 1

Conceptual model and framework of organic aquaculture



Source: Reproduced with permission under Creative Commons Attribution 4.0 International license. Beg, M.M., Roy, S.M., Ramesh, P., Moulick, S., Tiyasha, T., Bhagat, S.K., Abdelrahman, H.A. 2024. Organic Aquaculture Regulation, Production, and Marketing: Current Status, Issues, and Future Prospects—A Systematic Review. *Aquaculture Research 1*, 5521188. https://doi.org/10.1155/2024/5521188

Xu *et al.* (2017) compared computer vision and hyperspectral imaging systems along with chemometric and machine-learning tools to rapidly differentiate organic and conventional fresh and chill-stored aquaculture salmon fillets. The best prediction performance was observed when the analytical results of the hyperspectral imaging in the 400–1000 nm region were processed with the support vector machine (SVM) tool.

7.6 METHODS FOR THE DETECTION OF UNAUTHORIZED OR UNDECLARED PROCESSING PRACTICES

Fraud in the fisheries and aquaculture sectors can be linked to unauthorized or undeclared processing practices, such as treatment with carbon monoxide (CO); unauthorized or undeclared usage of additives, such as nitrates, nitrites, formaldehyde, or sulphites; or the addition of water and water-binding agents to increase weight.

7.6.1 Detection of the treatment of fish with carbon monoxide

Fish may be treated with carbon monoxide (CO) at different stages, from fresh catch to slaughtering, distribution, pretreatment before storage, processing, or packaging. The purpose of this treatment is to enhance and prolong the red colour, by preventing discoloration caused by myoglobin and haemoglobin oxidation, and to reduce lipid oxidation. Treatment of fish rich in red muscle such as tuna (*Thunnus* spp.)

and mahi-mahi (*Coryphaena hippurus*) with filtered smoke generated from natural sawdust after the removal of undesirable taste and odour components, carcinogenic compounds and gases, is the dominant commercial technology. Fish may also be pretreated, packaged or stored in high concentrations of CO (Concollato *et al.*, 2015). However, this practice is prohibited in several countries, mainly due to microbiological risks arising from masking fish spoilage, but also due to potential toxic effects of CO. Fraudulent use of CO can particularly compromise the safety of histidine-rich fishes such as tuna, mackerel, sardine, herring and swordfish, as it can result in the formation of histamine from the oxidative decarboxylation of histidine (Djenane and Roncalés, 2018).

Gas chromatographic and spectroscopic techniques are currently being used to detect the unauthorized or undeclared processing of fish with CO.

7.6.1.1 Gas chromatographic methods

Gas chromatography (GC), coupled with flame ionization detection (FID) or with mass spectrometric (MS) detection, has proven very sensitive in quantifying CO residues in fish.

In the GC-FID technique, the components of the sample, after their separation in the GC column according to their physicochemical properties (such as their volatility), pass into a hydrogen/air flame, where they become ionized within the flame. The ions are then collected by electrodes, creating a small current that is converted into an electrical signal, which is measured. Chow *et al.* (1998) successfully employed a GC-FID system equipped with a reducing column after the main column to change CO to methane before entering the FID to determine the CO residue in tuna flesh. This approach improved the sensitivity by 200 times over that of using GC without a reducing column.

GC-MS allows for the accurate identification and quantification of the components of complex samples by separating them and measuring their mass-to-charge ratio. Headspace gas chromatography coupled with mass spectrometry (HS-GC-MS) involves the extraction of CO into a headspace vial and subsequent analysis of the headspace gas, allowing for more sensitive determination of CO in fish. Anderson and Wu (2005) employed this technique for the quantitative determination of CO in tuna and mahi-mahi tissues. The difference between untreated and treated specimens was in the range of an order of magnitude. The same technique has been applied to improve the detection of the treatment of fish meat of tuna, yellowtail and tilapia with CO, which is not allowed in Japan. Through an interlaboratory study, it was revealed that the CO level of many samples of tilapia exceeded the regulatory maximum limit, which had not been observed when using an alternative method (Ohtsuki *et al.*, 2011). The usage of a programmed temperature-vaporizing (PTV) injector upon HS injection and the restoration of the GC column by oven temperature programming boosted the robustness of an HS-GC-MS method developed by Bartolucci *et al.* (2010) for the determination of CO in tuna fish. The level of CO in treated samples differed markedly from that detected in the untreated ones.

7.6.1.2 Spectrophotometric methods

A simple and rapid analytical tool for the quantitative determination of the adduct of CO with myoglobin (CO-Mb) in tuna is provided by UV-Vis spectrophotometry and analysis of electronic absorption spectra, particularly regarding the characteristic Soret band at 420 nm. However, comparison of the results with those obtained by HS-GC-MS revealed that the UV-Vis spectrophotometric method underestimates the amount of total CO, as it mainly detects only CO bound to the iron (Fe) atom of

the haeme protein. Despite this limitation, the spectrophotometric method provides a low-cost, rapid-screening control approach (Droghetti *et al.*, 2011; Smulevich *et al.*, 2007).

The HS-GC-MS and GC-FID methods are more expensive and generally provide higher sensitivity and lower detection limits compared to spectroscopic methods. The selection of an appropriate method for the detection of treatment of fish with CO depends on the requested level of accuracy, rapidity, equipment availability and cost, as well as the screening or confirmatory purpose of the analysis.

7.6.2 Detection of the treatment of fish with nitrate or nitrite

Nitrates and nitrites are ubiquitously present in nature as part of the nitrogen cycle, and they occur in all organisms as part of the endogenous nitrate-nitrite-nitric oxide pathway. They are also considered environmental contaminants, emitted from industrial, agricultural and urban activities. Sodium and potassium nitrates and nitrites are authorized food additives in some food categories for preservation purposes and for the fixation of colour and flavour – at specified maximum permitted levels. Their antibacterial action is attributed to the formation of nitric oxide following the progressive conversion of nitrates into nitrites (Vlachou *et al.*, 2020a; 2020b). The enhancement of red colour is attributed to nitric oxide, formed through the acidic treatment of nitrite, which binds as a ligand to myoglobin (Niederer *et al.*, 2019). Excessive levels of nitrate or nitrite in food may induce adverse health effects, mainly from nitrite, due to haematological and cardiovascular effects and the potential for the formation of carcinogenic nitroso compounds. Nitrate is considered of concern because of its reduction into nitrite. According to European legislation, only sodium and potassium nitrates can be used as additives for processed fish and fishery products, and specifically only in pickled herring and sprat. Additionally, their usage must be declared on the product label, in accordance with Regulation (EC) 1333/2008 (EU, 2008) and Regulation (EU) 1169/2011 (EU, 2011).

Detection of unauthorized or undeclared usage of nitrate or nitrite salts as preservatives in fish can be implemented with a variety of tests. The interpretation of the analytical results may be challenging and should consider nitrate and nitrite background levels because of their natural occurrence or because of environmental contamination.

7.6.2.1 Colorimetric and spectrophotometric methods

The Griess test is a rapid, low-cost test to detect the presence of nitrite ions in food. Detection is based on the formation of a red-pink colour of diazonium salts formed by the treatment of aromatic amines with nitrous acid produced by nitrite under acidic conditions. Nitrates can also be detected, after their reduction into nitrites on a cadmium column. Calculation of the nitrate concentration is then obtained by the difference. Quantification of nitrates and nitrites can be obtained by spectrophotometry (Sen and Donaldson, 1978; Moorcroft *et al.*, 2001).

7.6.2.2 Chromatographic methods

Gas chromatography coupled with flame ionization (FID) and electron capture detection (ECD) has been used by Toyoda *et al.* (1978) for the determination of nitrate in fish sausage and in cod and salmon roe. Nitrite was oxidized to nitrate with permanganate in the presence of sulphuric acid and chromatographed as nitrate. Sasaki *et al.* (2018) quantified nitrite in fish by liquid chromatography with UV detection after the extraction and clean-up of the samples by dialysis in a tris hydroxymethyl aminomethane solution.

Chiesa *et al.* (2019) developed methods for the determination of nitrate in seafood (fish, shrimp and bivalve species) based on ion chromatography with suppressed conductivity. Significant differences in nitrate concentrations were observed between farmed and wild seafood species, with the highest concentrations found in smoked-salmon samples. Nitrite was not detected in any sample.

Headspace-gas chromatography-mass spectrometry (HS-GC-MS) was employed by Niederer *et al.* (2019) in a study that revealed that 45 percent of all tuna samples that were taken from the Swiss market had been illegally treated with nitrite. The method is based on the two-step reduction of nitrite to nitric oxide, which is then reduced to nitrous oxide. The method was validated using 15N labelled nitrite as well as treated and untreated reference fish samples.

7.6.3 Detection of the treatment of fish with formaldehyde

Illegal treatment of fish and seafood with formaldehyde to extend their shelf life is a common problem reported in many countries. Low levels of formaldehyde may occur in fish muscle as a product of the endogenous trimethylamine oxide (TMAO) degradation pathway. Endogenous production of formaldehyde can increase due to improper storage conditions (Jinadasa *et al*, 2022). However, excessive occurrence raises health concerns, as formaldehyde has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2006).

7.6.3.1 Titration

The classical sulphite titration assay for the analysis of formaldehyde has been further developed in paper-based analytical devices. Formaldehyde reacts with excess sulphite, and the generated sodium hydroxide is quantified on the device using acid-base titration with sulphuric acid and phenolphthalein as the indicator (Taprab *et al*., 2019; Tasangtong *et al*., 2022). Microwell plate titration with fabrication-free, ready-to-use plates allowed for easily portable semiquantitative onsite analysis of formaldehyde in shrimp, squid, oyster and jellyfish in Thailand (Tongdee *et al*., 2024).

7.6.3.2 Colorimetric and spectrophotometric methods

Digital image colorimetry has been used for rapid and low-cost detection of formaldehyde in fish and squid samples, using a biodegradable colorimetric film. The detection was based on the entrapment of colorimetric reagents within a thin film of tapioca starch and the formation of a yellow reaction product (Wongniramaikul *et al*., 2018).

Spectrophotometry has been employed to quantify formaldehyde residues in formalin-treated farmed olive flounder (*Paralichthys olivaceus*) and black rockfish (*Sebastes schlegeli*) after bath treatment with formalin. This method included a wet-chemistry sample-preparation procedure, and the absorbance of the violet colour was read at 550 nm (Jung *et al*., 2001). A rapid, simple, multisample method was developed by Weng *et al.* (2009) for detecting formaldehyde using a heated (polydimethylsiloxane) microfluidic chip with multiple reaction reservoirs and measuring the absorption rate at 410 nm.

7.6.3.3 Sensors

Sensor technology provides rapid, low-cost detection of hazards in food, opting for onsite measurement. A variety of sensing technologies to determine exogenous and endogenous formaldehyde levels in fish have been developed to reveal illicit addition or improper storage. Sensing mechanisms are based on small fluorophores, nanomaterials, polymers, or metal frameworks (Roy *et al*., 2024).

Rapid onsite analysis of trace formaldehyde in squid and shrimp samples has been achieved by surface-enhanced Raman spectroscopy (SERS) after purge-sampling and

derivatization steps. Au/SiO2 nanoparticles were employed for enhancing the Raman signal intensity (Zhang *et al*., 2014).

An electrochemical sensor allowed for fast and reproducible quantification of formaldehyde in Malabar red snapper (*Lutjanus malabaricus*) and longtail tuna (*Thunnus tonggol*) with the differential pulse voltammetry (DPV) method. The analytical system set-up comprises the usage of an ionic liquid, gold nanoparticles, chitosan and glassy carbon electrodes. Methylene blue was used as a redox indicator to increase the electron transfer in the electrochemical cell. The developed biosensor measured the nicotinamide adenine dinucleotide electron from the NAD+ reduction at a potential of 0.4 V (Noor Aini *et al*., 2016).

Based on the ability of formaldehyde to alter laser light reflection properties in contaminated food samples, Yasin *et al*. (2019) developed a fibre-bundle sensor, which allows the non-destructive detection of formaldehyde in snapper and the gourami fish in Indonesia, employing red laser light at 630 nm.

7.6.3.4 Chromatographic methods

Wahed *et al*. (2016) used HPLC for the detection of formaldehyde in fresh fish and fish feed collected from local markets in Bangladesh.

Gas chromatography coupled with mass spectrometry (GC-MS) has been employed for the determination of formaldehyde in 12 species (sea fish, freshwater fish and crustaceans), following sample preparation with solid phase microextraction and fibre derivatization with pentafluorobenzyl-hydroxyl-amine hydrochloride. Fish belonging to the *Gadidae* family exhibited the highest formaldehyde concentration (Bianchi *et al*., 2007). Separation of formaldehyde with a MoO_3 /polypyrrole intercalative sampling adsorbent and consecutive measurement with GC-MS allowed for the quantification of formaldehyde at trace levels in aquatic products (Ma *et al*., 2015).

7.6.3.5 Spectroscopic methods

Infrared and near-infrared spectroscopy can be used to detect formaldehyde in fish. Detection is based on observed changes in the infrared spectrum of treated fish samples, particularly in spectral regions related to protein structure. A Tri-step IR method combined with partial least squares (PLS) regression analysis has been proven suitable for the quantitative determination of formaldehyde in squid (Gu *et al*., 2017). Ellegaard Bechman and Jørgensen (1998) determined formaldehyde in the skin of 115 thawed, whole Atlantic cod samples with a combination of the evaluation of near-infrared diffuse reflectance spectra and PLS regression.

7.6.4 Detection of the treatment of fish with sulphur dioxide or sulphites

Sulphur dioxide and sulphites are used as food additives due to their antibacterial activity and technological functions, including bleaching and colour enhancement. For example, they have long been used as a treatment to prevent prawn and shrimp melanosis (blackspot) (Bonerba *et al*., 2013). Inhalation and oral or dermal exposure to sulphur dioxide and sulphites can induce hypersensitivity (immunologically initiated – allergy) and intolerance (non-immunologically triggered – pseudo-allergy) reactions, manifested as respiratory, dermatologic, or gastrointestinal symptoms. Additionally, there is some toxicological evidence from animal studies that associates sulphite exposure with reproductive and developmental toxicity (EFSA, 2022).

In the European legislation, sulphur dioxide and sulphites as additives are prohibited in unprocessed fish and, in processed fish, they are only authorized in dried, salted fish of the *Gadidae* species. Sulphur dioxide and sulphites are authorized for usage in unprocessed (fresh, frozen and deep-frozen) and in cooked crustaceans and cephalopods, in accordance with Regulation (EC) No 1333/2008 (EU, 2008).

7.6.4.1 Distillation – alkali titration

A commonly used protocol for the analysis of sulphur dioxide and sulphites in food is the Monier-Williams method, which comprises extraction of SO_2 by heating with phosphoric acid and the production of sulphuric acid in the presence of hydrogen peroxide. Sulphuric acid is then titrated against sodium hydroxide (Yamagata and Low, 1992; Hardisson *et al.*, 2002). This method, which does not require high-cost laboratory equipment, has been successfully applied for the determination of the content of sulphite in prawns and shrimps (Williams *et al.*, 1990; Hardisson *et al.*, 2002).

7.6.4.2 Colorimetric methods

Ogawa *et al.* (1979) obtained more rapid and sensitive sulphite quantification in shrimp using a modified distillation apparatus and replacing the alkaline titration with pararosaniline colorimetry, which also achieved higher recovery performance. Currently, commercial analytical systems exist, which use colorimetric test strips for semiquantitative sulphite analysis in food.

7.6.4.3 Polarographic methods

A differential pulse polarographic method developed by Holak and Patel (1987) applied a modified Monier-Williams distillation with a strongly acid solution. SO_2 is trapped by purging with an acetate buffer and then polarographed. The method has been proven suitable for analysing sulphites in shrimp, with recoveries comparable to those for the official Monier-Williams method at high levels and with superior recoveries at low levels. Stonys (1987) also used the classical Monier-Williams distillation followed by square wave voltammetry for quantifying sulphites in shrimp, a rapid approach that is very sensitive and specific for SO_2.

7.6.4.4 Distillation iodometry

This methodology involves the conversion of sulphites into sulphur dioxide, which is then transferred through steam distillation into a standard solution of iodine. After the redox reaction between the sulphur dioxide and the iodine is completed, the residual iodine is determined by redox titration, using a standard solution of sodium thiosulfate. Iodometric titration is preferred because it is more selective for sulphur dioxide and avoids interference from other volatile acids present in the sample (Vyncke, 1991; 1992).

7.6.4.5 Chromatographic methods

Williams *et al.* (1990) developed analytical methods for the determination of the sulphite content in shrimps and prawns employing an HPLC system with electrochemical detection (ECD) fitted with a platinum electrode. The results were in close agreement with those obtained through the Monier-Williams method. Additional advantages were shorter analysis time and a much smaller sample required. High-performance liquid chromatography, coupled with fluorescence detection, was used to quantify the sulphur dioxide content in squid, after a derivatization reaction forming the fluorescent 2H-isoindole-1-sulfonate (Mu *et al.*, 2022). Employment of the headspace gas-liquid chromatography technique with flame photometric detection has quantified SO_2 levels in shrimp, with results comparable to those obtained with the Monier-Williams and the colorimetric techniques (Mitsuhashi *et al.*, 1979).

The combination of a modified Monier Williams distillation with ion chromatography, coupled with conductivity or electrochemical detection, provided sensitive and selective sulphite determination in shrimp (Sullivan and Smith, 1985; Anderson *et al.*, 1986).

A method developed by Iammarino *et al.* (2010), based on ion chromatography and suppressed conductivity detection, offers accuracy, precision, speed and automation, without the need of laborious sample-preparation procedures.

7.6.4.6 Electrophoretic methods

A rapid method for the quantification of sulphites in seafood has been developed via capillary-zone electrophoresis with indirect UV-Vis detection. This method was successfully applied to quantify sulphites in shrimps from the Brazilian market and revealed illicit usage of sulphites in some samples (Gonçalves *et al.*, 2020).

7.6.4.7 Flow injection analysis

Flow injection analysis offers rapid, accurate, low-cost and automated determination of sulphite in food. The method is based on the decolourization of malachite green by SO_2, which is extracted from shrimp with tetrachloromercurate (II) reagent and isolated from the flowing sample stream (Sullivan *et al.*, 1986; Ruiz-Capillas and Jiménez-Colmenero, 2009).

7.6.5 Detection of added water and water-binding agents in fish and fishery products

Water is a natural constituent of fish. Although data on natural water composition are not available for all fish species, it is considered that the natural water content in fish fillets can vary from 55 percent to 82 percent, depending on species, habitat and diet. Water can be added to fisheries products during processing – chilled or deep-frozen fish is usually covered with a protective glaze of ice. According to regulatory requirements, for example European Regulation (EU) No. 1169/2011 (EU, 2011), water content must be declared on the labelling. Loss of tissue water can occur, particularly in frozen, raw fish products. This is the rationale for the authorization of the usage of water-binding additives, such as condensed phosphates. However, a common type of fraud is the addition of significant amounts of water to unprocessed fishery products, sometimes in combination with approved and non-approved water-binding substances, to increase product weight and profit.

The assessment of added water in raw fish and fish products is complex. In 2024, the Fish and Fishery Products working group of the Working Group of Experts in the Field of Food Hygiene and Food of Animal Origin (Arbeitskreis der auf dem Gebiet der Lebensmittelhygiene und der Lebensmittel tierischer Herkunft tätigen Sachverständigen) of the German Federal Office of Consumer Protection and Food Safety issued a guideline entitled *Addition of Water in Unprocessed Fishery Products – Evidence and Assessment Options* (BLV, 2024). The guideline includes the following suggested parameters to be investigated in fish, crustaceans and molluscs:

- **Sensory parameters in raw and cooked states.** Comparison of appearance (morphology and water loss); consistency/haptics; taste and smell.
- **Protein content and water/protein ratio.** According to data from the literature, untreated fish fillets, as well as crustaceans and molluscs, contain between 15 percent and 25 percent crude protein, depending on the species, physiological state and diet. Protein contents of 15 percent and less in lean fish fillets (fat content <2 percent) might indicate added water. An appropriate investigation approach for this purpose is laboratory analysis to calculate the water/protein ratio and compare it with in-house or literature reference values based on authentic raw materials. If the calculated ratios for the respective sample under assessment are higher than those for untreated muscle, water addition can be assumed.

- **pH value**. Raw, untreated fish fillets typically have pH values below 7.0. This also applies for crab and mollusc muscle, although slightly alkaline pH may be observed for some species. Water-binding additives can result in changes in pH value. However, pH must be carefully considered along with the declared usage of acidifying or alkalizing substances.
- **Condensed phosphates, carbonate and citrate as water-binding substances**. These substances can be used in fish and fishery products, at various authorized levels, to prevent the loss of water from tissues, but they can also occur at low levels due to endogenous formation.
- **Total ash or salt-free ash**. According to literature data, the total muscle ash content of fishery products is around 1 percent. Higher levels may indicate the addition of inorganic components, while lower levels can indicate dilution with the addition of water.
- **Sodium chloride and sodium content**. Based on available literature, sodium levels in untreated fish muscle are in the range of 20 mg–160 mg/100 g, corresponding to NaCl contents of 0.1 percent to 0.25 percent. Typically, sodium and chloride ions are present in fish fillets in a stoichiometric ratio of approximately 1:1, which remains unchanged even when salt is used in the preparation. Excessive sodium levels can be detected in products treated with sodium salts (such as sodium citrate), suggesting the use of water-binding additives. It is suggested that the sodium chloride content be evaluated based on the measurement of the chloride content.
- **Potassium content**. Potassium levels in unprocessed fishery products are typically in the range of 100 mg–500 mg/100 g. Tissue damage caused by improper or repeated freezing can reduce potassium levels. A shift in the naturally expected sodium-to-potassium ratio in a sample, in addition to the absolute levels, is an indication of the use of sodium- or potassium-containing substances (as ingredients or additives).

Paul *et al.* (2012) demonstrated that the consideration of sensory parameters was effective in revealing the adulteration of giant freshwater prawn (*Macrobrachium rosenbergii*) by injecting tripolyphosphate and materials such as pearl tapioca (*sagu*) or jelly (*litchi*) before freeze processing for increasing weight.

The official method for the quantification of seafood fat-free protein is based on nitrogen determination and is also used to calculate nitrogen factors and to estimate the added water. Measurement of the nitrogen content can be done according to the classical Kjeldahl method or the rapid Dumas method, the latter measuring both protein and non-protein nitrogen (Thompson *et al*, 2002; Analytical Methods Committee, 2014).

Water content can be determined in seafood by a reference method based on loss in mass obtained after mixing the test portion with sand and drying to constant mass at 103 ± 2 °C (van Ruth *et al*., 2014). Nuclear magnetic resonance (NMR) has become a key analytical tool in fish authentication, including the water and protein content and water-holding capacity (Erikson *et al*., 2004; Erikson *et al*., 2012). Recent research has focussed on non-destructive rapid approaches for profiling water and protein in fish and seafood products. In a study from Xiaoyan *et al*. (2012), near-infrared spectroscopy technology and support vector machine (SVM) were employed to estimate surimi moisture and protein. Spectral imaging and statistical tool combinations allowed for the quantitative measurement of moisture and fat content and their spatial distribution in fish fillets from different species (ElMasry and Wold, 2008).

Bisenius *et al*. (2019) treated cod fillets with phosphates, citrates and carbonates at controlled conditions to increase the water content and to investigate the impact of the treatment on various parameters of the final product. Phosphate and citrate levels

were quantified using ion chromatography and carbonate with gas chromatography. The temperature and the duration of the treatment influenced all investigated parameters. Additionally, sensory aspects were additive dependent. Triphosphate, hydrogen carbonate, and the mixture of citrate and hydrogen carbonate showed the highest potential for water binding, while citric acid may not be appropriate as a sole water-binding substance. Adding food additives leads to changes in the pH value of fish meat. Treatment with hydrogen carbonates or triphosphate shifted the pH to the alkaline side, but the effect was clearer with carbonates. Thus, a fillet treated with hydrogen carbonate can easily be distinguished from an untreated one simply by measuring the pH. Additionally, Bisenius *et al.* (2019) reported that increases in pH often led to higher water content in fillets, and that treatment with citric acid induced the lowest pH. The authors also concluded that several parameters should be collectively considered to detect added water in fish products. The pH, the water/protein ratio and the *p* value (*p* value = $(P_2O_5(\%)*100)/(\text{protein}(\%))$ can be important indicators for the usage of additives, especially for carbonates and phosphates. To assess compliance with legal requirements and facilitate the assessment of added water and food additives, the knowledge of natural background levels of citrate, phosphate and carbonate – compounds that are part of the fish metabolism – is imperative. Furthermore, in a subsequent investigation, Bisenius *et al.* (2020) created reference values for water, protein and fat content of herring and cod fillets from different FAO fishing areas and quantified the naturally occurring carbonate and monophosphate contents in untreated fish fillets.

CHAPTER 8

Case studies of food fraud in the fisheries and aquaculture sector

Case study 1. Species identification by molecular tools in mussel products sold in the Italian market: major issues and future challenges

INTRODUCTION

FishLab (at the Department of Veterinary Sciences of the University of Pisa) was consulted by a wholesaler to solve an authentication issue concerning a batch of precooked frozen mussels labelled "Chilean mussels" (*Mytilus chilensis*). Indeed, the samples of the batch had been differently molecularly identified by two external labs: the first identified them as *M. chilensis*, using the 16Sr RNA gene as the molecular target, and the second identified them as *Choromytilus chorus*, using the COI gene. The COI gene was selected by FishLab for the analysis to compare their results with those previously obtained, even though, according to the literature (Larraín *et al*., 2018), the mitochondrial genes are not suitable for the identification of species belonging to *Mytilus* spp. The 16Sr RNA gene was not considered given its even lower interspecies variability degree. Additionally, the polyphenolic adhesive protein (PAP) gene, a nuclear marker reported as more suitable for *Mytilus* spp. identification, was considered and, as expected, the analysis allowed for the identification of samples at only the genus level (*Mytilus* spp.) using the COI gene. The PAP amplification results suggested the presence of Chilean mussel and/or Mediterranean mussel by electrophoretic run, based on the length of the fragments. However, based on the Phred quality score, the PAP sequences were not considered reliable, and a species-level identification was not achieved (Giusti *et al.*, 2020) FishLab decided to further investigate this topic and, in collaboration with Italian zooprophylactic institutes (official laboratories of the Ministry of Health), conducted a study applying a PCR-RFLP technique proposed by Santaclara *et al.* (2006) to market products (including some samples of the batch previously analysed). Correspondence with label information was also verified. The PCR-RFLP identified 47.2 percent of the products as Chilean mussel, 36.1 percent as Mediterranean mussel, 8.3 percent as a mix of pure species and hybrids, and 8.3 percent as hybrids. The labelling of all the products was compliant with labelling legislation in force in the European Union (Giusti *et al.*, 2022).

THE CASE IN THE LITERATURE

Morphological identification in mussel specimens is challenging due to phenotypic plasticity, and it is often impossible in processed shelled products, encouraging illegal practices of species substitution. In addition, hybridization between mussel species is reported in geographical areas where two or more species coexist (Larraín *et al.*, 2018.). In the European Union, hybrids Blue mussel (*M. edulis*) x Mediterranean mussel and Blue mussel x Pacific blue mussel (*M. trossulus*) are reported along the Atlantic coast and in the Baltic Sea, respectively. In Chile, hybrids Chilean mussel x Mediterranean mussel, Chilean mussel x Pacific blue mussel and Chilean mussel x Blue mussel have been detected (Giusti *et al*., 2022). Molecular studies identifying species

in mussel-based market products are scarce probably because the mitochondrial molecular targets used as a standard for seafood authentication are ineffective. In addition, mislabelling (that is, label non-compliance with law dispositions) has rarely been evaluated in the literature. However, Colihueque *et al.* (2020) recently highlighted a 50 percent mislabelling rate in products labelled Chilean mussel that were identified instead as black mussel (*Aulacomya atra*). In another study, authors detected one clear mislabelling case: a product sold in the Portuguese market as Chilean mussel but identified as Choro mussel (*Choromytilus chorus*) (Harris *et al.*, 2016). However, mislabelling data could be underestimated; on the one hand because there is insufficient data to produce useful estimates on mislabelling rates for all the invertebrate categories, and on the other hand probably due to issues in selecting the proper molecular targets for the identification of *Mytilus* spp. In this respect, a recent systematic review and meta-analysis investigating the mislabelling rate in seafood sold on the Italian market highlighted that this taxon is still insufficiently analysed to provide informative data (Giusti *et al.*, 2023b).

SCALE AND GLOBAL INCIDENCE OF THE CASE

Mussels (Bivalvia) are commercially relevant products in the European Union. The blue mussel and the Mediterranean mussel are the species mainly consumed and produced in the European Union, covering 85 percent of mussel production in 2018. A large part is consumed fresh, frozen, or canned; but processed, ready-to-eat products are also increasingly present on the market (Avdelas *et al.*, 2021). More than 90 percent of the national production takes place in Emilia-Romagna, Veneto, Apulia, Friuli-Venezia-Giulia, Sardinia and Liguria, and Mediterranean mussel is the main species produced. The production of this species, which is mainly sold fresh in Italy, is however not enough to meet the national consumption demand. Italian imports of mussels, mainly from Spain and Chile, reached 73 066 tons in 2017. Mediterranean mussel, blue mussel and Chilean mussel are the most-consumed species, with Mediterranean mussel and blue mussel produced in European Community waters and Chilean mussel imported from Chile. Mussels make up about three-fourths of Spanish aquaculture production, and Spain is by far the main producer and exporter of Mediterranean mussel, while, at the international level, Chile has recently become the world's second-largest producer and exporter of farmed mussels (after China). Chilean production is mainly based on the native blue mussel, although other Mytilidae species are also farmed, such as blue mussel, black mussel and choro mussel (FAO, 2022a; Avendaño *et al.*, 2017).

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

Mussels are filter-feeding organisms that have the potential to accumulate and concentrate a variety of marine toxins and pollutants into their flesh and can therefore pose a health risk to human consumers. Therefore, for human protection and as required by law, European Union Member States are obliged to conduct routine analyses from shellfish-harvesting sites (Commission Implementing Regulation, EU 2019/627). Tetrodotoxins (TTXs), a group of potent neurotoxins named after the Tetraodontidae fish family (puffer fish), have also been reported in bivalve molluscs farmed in the Pacific area and, recently, in European Union seas. Since 2015, several cases of TTXs shellfish contamination have been reported in Greece, the Kingdom of the Netherlands, Spain and the United Kingdom. More recently, the presence of TTXs was identified in mussels from Italian waters. In response to this, the European Union Food Safety Authority determined that a concentration of TTXs below 44µg/kg TTXs in shellfish meat, based on a large portion size of 400 g, would not entail adverse effects in humans (EFSA, 2017). Considering that mussels harvested in the European Union are subject to stricter control compared to products harvested outside the

European Union, the species may in part represent a sort of guarantee of security. Thus, the availability of analytical tools for verifying mussel traceability are necessary also in light of protecting consumer health in the face of such emerging risks.

TOOLS TO PREVENT THE ISSUE

The European Union has given great importance to guaranteeing that consumers make informed choices in relation to the food they consume and to preventing misleading practices. Therefore, appropriate methods are needed to deal with this issue. Recently, a collaboration with the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, allowed for further optimization of a sequencing protocol for *Mytilus* spp. identification using the PAP gene. Tissue samples were collected directly from production sites in Chile or from national markets. Additionally, some DNA samples already identified by PCR-RFLP in the above-mentioned study were used. The target PAP region was amplified from all the samples (DNA and tissue). All PCR products were sequenced using the Sanger technique. Forty-one percent of tissue samples were randomly selected to perform the RFLP analysis to be compared to the sequencing results. Overall, the species identification by PCR-RFLP failed for 9.4 percent of tested samples. These findings suggest that the optimized protocol relying on Sanger sequencing has some practical advantages over PCR-RFLP. Considering the decreasing costs of sequence-based technology, this sequencing protocol is proposed as a valid, consistent and reliable alternative to the methods currently used. Also, the applicability of next-generation sequencing technologies to species identification in mussels is under investigation.

The method should be further assessed in light of the new data through the production of a sufficient number of reference sequences from vouchered identified specimens. Indeed, database construction and validation represent the first step for all food-authentication methods.

CONCLUSIONS AND RECOMMENDATIONS

The adoption of a problem-solving approach to overcome unavoidable limitations of the DNA standard analytical procedures is required to ensure efficient support for the seafood-traceability system (Tinacci *et al.*, 2018b). Accurate interpretation of analytical results and the choice of the appropriate methodological approach are pivotal to ensuring an adequate and objective technical opinion. Method reliability is even more important if the results are to be used as acceptable evidence in a court of law. Not least, from the perspective of knowledge transfer and of sharing methods with other laboratories, the analytical protocol should be set up in as affordable a manner as possible, since most laboratories have access only to basic molecular-biology equipment. Despite the well-known presence of hybrid specimens in the market, also confirmed by the outcomes of our analyses, no indication of this is provided on the labelling at the level of the European Union, nor do they appear in any official list of commercial designation published by the Member States. This aspect, in addition to hindering the implementation of strategic-management plans aimed at preserving native populations and safeguarding the quality of aquaculture production, creates difficulties for food-business operators in guaranteeing food transparency. In this respect, a revision of the European Union labelling system is recommended. Until then, in the context of official-control or self-control activities, it may be reasonable that, where one of the parental species involved in the inbreeding process is reported in the label, the product could be considered compliant. This would help official and private laboratories in interpreting results and issuing reports. Harmonizing taxonomy in the context of aquaculture production, traceability, labelling and trade of Mytilus products is more complex compared to other seafood products. However, at the state of the art, Chilean mussel and Mediterranean mussel should both be simply labelled

as "*Mytilus sp*. - mussel", also considering that the origin declaration is mandatory according to the current seafood-labelling legislation, as established in Regulation (EU) No 1379/2013. In this way, consumers will be informed of the origin, even with the adoption of a more generic term. Moreover, considering the well-known presence of hybrid specimens in the market, it may be advisable to extend this more general nomenclature also to blue mussel.

Case study 2. Species identification in complex seafood matrices (fish burger) in the age of metabarcoding

INTRODUCTION

Complex seafood products (such as fish burgers, surimi, fillings, etc.) are particularly susceptible to fraudulent species substitution due to their nature, which makes it impossible to identify the species used as ingredients without relying on molecular analysis (Giusti *et al*., 2017). In the last few years, seafood companies have often asked FishLab, at the Department of Veterinary Sciences of the University of Pisa, to analyse these types of products by means of metabarcoding. This method involves using universal PCR primers to massively amplify one or more taxonomically informative targets, thus allowing for DNA barcoding in parallel, owing to next-generation sequencing technologies (NGS). This demonstrates that food-business operators (FBOs) are well inclined to include analyses relying on innovative molecular technologies in their self-control systems. In this study, the fish burger was selected as a model to implement a metabarcoding workflow on the Illumina platform for the authentication of complex seafood products in an efficient, reliable and easily transferable manner. The entire workflow was structured by setting up and analysing each analytical step (from sampling to bioinformatic analysis and data interpretation), and experimental samples (positive controls, analytical blanks, replicates and samples processed out of the fume hood) were included to assess quality control throughout the process. Twenty-four sample replicates from nine products declared as European seabass were processed, together with 16 experimental samples (total of 40 samples processed). A ≈200 bp region of the *16Sr RNA* gene was selected as the molecular target. The sequencing was performed by an external company, and the data obtained were processed using the DADA2 R package. The taxonomic assignment was performed using Blastn (the NIH's local alignment search tool for nucleotides) against GenBank (identity value ≥99percent). The total number of reads ranged from 25 006 to 264 841. Differences in the number of reads are related to the fact that samples were sequenced in different runs. After the data processing, the percentage of maintained reads for each sample ranged from 73.8 percent to 96.8 percent. The sequences assigned to European seabass were highly predominant in all the products, with percentages ≥99.34 percent, except for one, where also a high number of sequences assigned to Atlantic salmon were found (12.41 percent). Sequences identified with other species (seafood, mammals, insects) were ≤0.57 percent, and in 14 percent of the cases they did not achieve even 0.001 percent. A threshold value of 3.3 percent to remove false positives was fixed, based on the results of the positive controls. According to the interpretation of the results, the laboratory procedures contributed minimally to the presence of contamination, which instead seemed to originate from previous phases in the production chain. Overall, metabarcoding proved an effective technique to assess the ingredients contained in complex seafood products. However, further investigation, including a higher sample number and interlaboratory validation, should be performed to validate the procedure (Giusti, Malloggi, Lonzi *et al*., 2023b)

THE CASE IN THE LITERATURE

Illumina-based massively parallel sequencing, with its ability to simultaneously sequence all the DNA molecules in the same sample (including those present in trace amounts), represents the most promising analytical tool to authenticate complex seafood products that may contain a wide range of species (such as minced matrices composing burger, surimi, fillings, etc.) (Haynes *et al.*, 2019). Despite this, its application in foodstuffs is limited. On the contrary, in other fields of research, such as the analysis of biodiversity in environmental samples or the study of trophic interactions, metabarcoding is well developed and more widely used. This is probably linked to the lack of standardized protocols comprising the entire workflow (from the sampling to the final metadata interpretation), which can make the analysis complex, lengthy and costly, without guaranteeing reliable final outcomes, thus discouraging its application by laboratories. Recently, a systematic review was published to answer the question "Is the metabarcoding ripe enough to be applied to the authentication of foodstuff of animal origin?" (Giusti *et al.*, 2024). The scientific papers were analysed with respect to the metabarcoding phases, namely library preparation, sequencing and final data analysis. In addition, the papers were scored based on the use of quality-control measures (procedural blanks, positive controls, replicates, curated databases and thresholds to filter the data) (Giusti *et al.*, 2024). It was observed that only 23 included papers were published since 2017. A lack of standardized protocols, especially with respect to the target barcode(s) and the universal primer(s), and the infrequent application of the quality-control measures, indicate that metabarcoding is not ripe enough for authenticating foodstuff of animal origin, although the observed trend in quality improvement over the years is encouraging (Giusti *et al.*, 2024)

SCALE AND GLOBAL INCIDENCE OF THE CASE

The global impact of food fraud is encouraging food companies to invest more in means and tools to prevent, manage and reduce this phenomenon. Cases of mislabelling involving complex seafood products are reported worldwide (Carvalho *et al.*, 2017; Giusti *et al.*, 2022). Moreover, the few available studies applying metabarcoding to complex seafood products found DNA from a very wide range of species, some not even seafood. This is of particular concern to FBOs, even more so because these products have currently won a large market share, following the demands of consumers who are increasingly seeking ready-to-eat and ready-to-cook products. The availability of an efficient and standardized metabarcoding protocol to detect fraudulent substitution in these products is required to reduce economic losses and guarantee maximum market transparency – a condition of consumer confidence.

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

The availability of an efficient molecular method to properly authenticate complex seafood products can also represent a tool to protect public health in case of the illicit presence of toxic species or the omission of potentially allergy-causing ingredients (such as crustaceans and molluscs), as already reported in the literature. In this respect, the presence of undeclared cephalopods (molluscs) or avian DNA (probably related to the presence of eggs) in surimi-based products and fish burgers is reported in the literature (Giusti *et al.*, 2017; Mottola *et al.*, 2022; Piredda *et al.*, 2022). Moreover, the presence of mammalian or avian species, also already reported in the literature, can represent a critical point for consumer protection based on religious or ethical issues. Furthermore, the possible presence of human DNA or DNA from insect pests could also be addressed using metabarcoding to evaluate FBO compliance with hygiene criteria. Finally, transparency in the market is a crucial point in safeguarding marine environments from illegal fishing practices, and in preventing the recycling of bycatch or fish waste.

TOOLS TO PREVENT THE ISSUE

Metabarcoding has been demonstrated to be an efficient tool for the authentication of complex seafood products. However, based on the outcomes of this study, further investigation should be performed in order to define standard operating procedures and harmonize protocols. Although the selected 16Sr RNA primer pair proved to be a good candidate for use with complex seafood products, additional performance tests on targeted species should be performed and the possibility of resorting to a multitarget/multigenic approach should not be excluded. In addition, the threshold value to exclude contamination should be further investigated considering factors such as the type of product, the species-diversity index and, not least, the different affinities of the primer pair with respect to the target species.

CONCLUSIONS AND RECOMMENDATIONS

Many of the issues encountered in this study have already been highlighted in literature dealing with food authentication using metabarcoding, but there is a lack of methodological studies and systematic reviews addressing all these aspects together. Therefore, it is recommended that standard operating procedures be defined to overcome these obstacles. It is pivotal to harmonize protocols by adopting quality-control measures that should be applied during the analysis. This, in turn, would allow for more extensive use of metabarcoding in the context of both official control by competent authorities and FBO self-control, thereby increasing the capability to detect and deter food fraud.

Case study 3. Fish mislabelling in Buenos Aires Province, the largest seafood market in Argentina

INTRODUCTION

In recent years, the vulnerability of fish products to fraud has multiplied. The fishing sector is recognized as one of the sectors most exposed to fraudulent practices, and governments are aware of the need to improve systems to control traceability and labelling within the fish supply chain (Tamm *et al*., 2016). However, the absence of policies governing fish and shellfish labelling and the improper application of such policies in some countries (Barendse and Francis, 2015; Miller *et al*., 2012; Xiong *et al*., 2016), along with other aggravating factors, such as the increase in world trade of fish, particularly processed products (Armani *et al*., 2015) and the depletion of fishery resources (Marko *et al*., 2004; Miller and Mariani, 2010), exacerbate this fraudulent behaviour. Proper food labelling is important for legal, health and environmental reasons. In addition, food-safety concerns are driving the need for accurately labelled food products, especially fish products that are indistinguishable solely on the basis of their morphology, such as fillets. Substitution of fish species or mislabelling (for instance, using a trade name which does not correspond to the actual species used to make the product) has multiple effects. Seafood mislabelling and substitution can have a number of consequences for consumers and the environment, including economic loss due to potential commercial fraud (Carvalho *et al*., 2017; Hanner *et al*., 2011; Von Der Heyden *et al*., 2010), public health effects (Chang *et al*., 2008), uncontrolled impacts on threatened fish species (Ardura *et al*., 2011) and damage to populations due to overfishing (Tokeshi *et al*., 2013).

THE CASE IN THE LITERATURE

Regarding economic issues, fishery products have shown high rates of species substitution, where species of higher commercial value are substituted with species of lower value, resulting in financial loss for the buyers and consumers. When a highly

prized species becomes overfished, retailers may be tempted to replace it with a common species of lower commercial value, resulting in commercial fraud (Pepe *et al.*, 2007). Fraudulent commercialization of fishery products has been reported in several countries in South America (Veneza *et al.*, 2018), North America (Hanner *et al.*, 2011), the European Union (Mariani *et al.*, 2015), Africa (Cawthorn *et al.*, 2012) and Asia (Chang *et al.*, 2016) (Table 4). Some traders may deliberately use mislabelling as a means to launder illegally caught fish into legitimate markets, or simply to defraud consumers for the purpose of accruing greater profits (Ogden, 2008). It is very important to consider the effect of these practices on threatened species, since fish retailers may offer endangered species, or species prohibited for international trade, for sale. In fact, unsustainable fishing pressure has led to the decline of most shark populations, with some shark species facing extinction (Dulvy *et al.*, 2008; Ferretti *et al.*, 2008; Worm *et al.*, 2013). These predators play a crucial ecological role in structuring marine ecosystems and food webs (Libralato *et al.*, 2006) and are commercially important for their meat (particularly their fins). Late maturation, low fecundity and longevity make sharks acutely vulnerable to overexploitation and prevent rapid recovery from overfishing (Stevens, 1999). Recent global catch assessments estimate that approximately 100 million sharks are landed annually, excluding IUU shark catches (Worm *et al.*, 2013).

TABLE 4
Examples of substitution rates reported for fish fillets

Country	Substitution rate (%)	Taxonomic focus	Detection method	References
Argentina	21.3	Diverse	DNA barcoding	Delpiani *et al.*, 2020
Brazil	17.3	Diverse	DNA barcoding	Carvalho *et al.*, 2017
Brazil	22	*Lutjanus purpureus*	DNA barcoding	Veneza *et al.*, 2018
Canada	41.2	Diverse	DNA barcoding	Hanner *et al.*, 2011
Canada	41.2	Diverse	DNA barcoding	Hanner *et al.*, 2011
France	3.7	Diverse	DNA barcoding	Bénard-Capelle *et al.*, 2015
Ireland	25	Cod	DNA barcoding	Miller *et al.*, 2010
Ireland	28.2	Cod	DNA barcoding	Miller *et al.*, 2012
Italy	32	Diverse	DNA barcoding and Cyt. b	Filonzi *et al.*, 2010
Italy	37.5	Diverse	DNA barcoding	Pappalardo and Ferrito, 2015
Italy	77.8	*Mustelus sp.*	DNA barcoding	Barbuto *et al.*, 2010
South Africa	21	Diverse	DNA barcoding	Cawthorn *et al.*, 2012
South Africa	50	Diverse	16S rDNA	Von Der Heyden *et al.*, 2010
South Africa	50	Diverse	16S rDNA	Von Der Heyden *et al.*, 2010
Taiwan Province of China	70	Diverse	DNA barcoding	Chang *et al.*, 2016
(the) United Kingdom	7.4	Cod	DNA barcoding	Miller *et al.*, 2012
(the) United States and Canada	25	Diverse	DNA barcoding	Wong and Hanner, 2008

SCALE AND GLOBAL INCIDENCE OF THE CASE

Chondrichthyans are regarded as the most threatened marine fish group in the world (Davidson and Dulvy, 2017). Moreover, overfishing has a deep, negative impact on them due to their biological characteristics. In the Southwestern Atlantic, these species are subject to commercial fishing as an incidental capture and are also globally subject to commercial, artisanal and recreational fishing (Bornatowski *et al.*, 2014; Chiaramonte, 1998). In 2022, in Argentina, 406 751.8 tonnes of shark were landed (Ministerio de

Economía, n.d.). Hence, there is an urgent need for proper food labelling to safeguard against legal, health and environmental issues. Currently, several countries such as Brazil and South Africa, as well as the European Union, have legal frameworks and governmental regulatory programmes that require appropriate species traceability and labelling (Filonzi *et al.*, 2010; South Africa, 2010). However, no regulations for seafood-product species identification exist in Argentina. In addition, until 2019, no studies of the potential substitution of high-value fish species for those of lower value, which is a common practice in several countries, had been conducted in Argentina (Delpiani *et al.*, 2020).

The evaluation of mislabelling in Argentina was carried out in three stages, resulting in three case studies: the first in coastal cities of the province of Buenos Aires; the second in the largest cities in the country; and the third in the coastal cities of Argentine Patagonia, thus covering the country's entire coastline. The evaluation was performed on the coast of Buenos Aires, since 75 percent of the fishing fleet is in the ports of Buenos Aires (Mar del Plata, Puerto Quequén, Bahía Blanca and General Lavalle), representing 70 percent of the landing of marine fishes in Argentina. The area includes 11 coastal cities of the Province of Buenos Aires (from north to south: San Clemente del Tuyú, Santa Teresita, San Bernardo, Pinamar, Villa Gesell, Mar del Plata, Miramar, Necochea, Claromecó, Monte Hermoso and Bahía Blanca), where 172 fish fillets were obtained, 164 of which could be sequenced, representing 28 species. Thirty-five cases of mislabelling were found, indicating an overall substitution rate of 21.34 percent. Thirteen cases involved substitution with bony fish, and 22 of the replacements involved chondrichthyans.

TOOLS TO PREVENT THE ISSUE

DNA barcoding is a powerful tool for rapidly determining the taxonomic group of a given organism. It can be used to discriminate between closely related taxa (Stoeckle *et al.*, 2004), it is easily comparable across different studies (Cline, 2012), and it can be used as a universal tool for food traceability. DNA barcoding based on the mitochondrial cytochrome c oxidase I (COI) gene is used to identify patterns in the mislabelling of the fishery products (Munguia-Vega *et al.*, 2022). DNA extraction, PCR amplification and sequencing of the COI gene were carried out following standard DNA-barcoding protocols as described by Ivanova *et al.* (2006, 2007).

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

In Argentina, there is no regulation or enforcement measures to ensure accurate labelling of seafood species. The sale of substituted or inaccurately labelled seafood can lead to various consequences for both consumers and the environment. These consequences range from financial loss due to possible commercial deception (Hanner *et al.*, 2011; Cutarelli *et al.*, 2014; Pappalardo and Ferrito, 2015; Carvalho *et al.*, 2017), to public-health risks (Chang *et al.*, 2008; Todd, 2011; Raimann *et al.*, 2014), and to unregulated harm inflicted on vulnerable fish species (Stevens *et al.*, 2000; Ardura *et al.*, 2010, 2011); this in addition to population declines caused by excessive fishing (Tokeshi *et al.*, 2013).

Substitution or mislabelling may pose serious health threats due to the potential presence of toxic, allergenic, or unidentified harmful substances (Holmes *et al.*, 2009; Ward *et al.*, 2008; Wong and Hanner, 2008). Ambiguous labelling further complicates the ability of consumers to avoid species that are at greater risk of extinction or that may trigger specific health concerns. Of particular concern is the impact of such practices on endangered species, as some vendors might unknowingly or deliberately sell threatened species or those banned from international trade. In addition, by purchasing these products, consumers may unintentionally contribute to irresponsible marketing practices (Holmes *et al.*, 2009; Wong and Hanner, 2008; van Leeuwen *et al.*,

2009). This is especially relevant for chondrichthyan species (including sharks, rays, skates and chimaeras), nearly 25 percent of which are classified as threatened (Dulvy *et al*., 2014). Accurate labelling serves not only conservation and legal purposes but also consumer safety and public health (Delpiani *et al*., 2024).

CONCLUSIONS AND RECOMMENDATIONS

Mislabelling can occur accidentally, probably linked to inaccurate morphological identification of fish, or it can be deliberate. The sale of southern eagle ray *Myliobatis goodei* as a skate species, or of the narrownose smooth-hound *Mustelus schmitii* as tope shark *Galeorhinus galeus*, may be accidental mislabelling, most likely due to the resemblance between these chondrichthyan species. On the other hand, inaccurate common names are sometimes deliberately applied to species in an attempt to increase sales by making the product more appealing to consumers. For instance, the name *pollo de mar* (the local common name for elephant fish *Callorhinchus callorhynchus*, whose real common name is *pez elefante*; similarly, *perita* was used to identify the southern kingcroaker *Menticirrhus americanus,* instead of its proper vernacular name, *burriqueta*. The names *salmonada*, *mora* and *vacío de mar* were used for the black drum *Pogonias courbina*; *palomito* was used for tope shark*;* and *palo rosado* was used for narrownose smooth-hound and for other sharks. The latter case causes confusion because narrownose smooth-hound was sold in the market as *palo rosado*, which is its corresponding popular name, but the official common name is *gatuzo*. This problem also becomes visible in cases in which different species belonging to the same genus or family are grouped under the same common name (Barbuto *et al*., 2010; Cawthorn *et al*., 2012), further diluting specific identification, such as happens with flounders, silversides or skates.

Another reason for mislabelling is the economic incentive of replacing higher-value species with lower-value species, as is the case in replacing cheeks of the pink cusk eel *Genypterus blacodes* (valued at USD 12/kg) with meat of the endangered spotback skate *Atlantoraja castelnaui* (valued at USD 3.80/kg) and the narrownose smooth-hound (valued at USD 5.50/kg). In addition, tope shark (valued at USD 4.50/kg) is sold as Argentine seabass *Acanthistius patachonicus,* (valued at USD 9.60/kg); elephant fish (valued at USD 4.25/kg) and narrownose smooth-hound are sold as yellow fin tuna (valued at USD 7.70/kg); and as Patagonian seabass *A. acanthitius patachonicus* (valued at USD 13) is sold as wreckfish *Polyprion americanus* (valued at USD 12.60/kg). The percentage of mislabelling of fillets obtained in the study is comparable to the results obtained in other studies carried out in Brazil (Carvalho *et al*., 2017), North America (Logan *et al*., 2008; Marko *et al*., 2004; Wong and Hanner, 2008), South Africa (D.M. Cawthorn *et al*., 2012) and the European Union (Barbuto *et al*., 2010; Bénard-Capelle *et al*., 2015; Filonzi *et al*., 2010; Miller and Mariani, 2010; Pappalardo and Ferrito, 2015), further demonstrating that this problem is widespread. According to the general results obtained, almost 70 percent of the replacements involved Chondrichthyes: mainly elephant fish, narrownose smooth-hound, tope shark, spotback skate and angel shark *S. Guggenheim* being sold as something else. This pattern is somewhat predictable, as in all surveyed fish retailers, the same triad of species was consistently available: "tuna" (mainly *Mustelus schmitti* and *Galeorhinus galeus*), "chicken fish" (*Callorhinchus callorynchus*), and common hake (*Merluccius hubbsi*). While the first two are Chondrichthyes, the common hake represents the most heavily exploited fish species in Argentina. Therefore, the fact that these elasmobranchs are now being marketed alongside hake suggests that previously less exploited species are being increasingly targeted. This trend may indicate that their populations are becoming subject to unsustainable fishing pressure, potentially following a similar trajectory of depletion to that experienced by hake. The increasing trend observed in shark-meat trade in many countries suggests that underlying demand for these

products, such as fins, fillets or shark oils, is increasing (Dent and Clarke, 2015). South Atlantic shark populations are facing intense fishing pressure (Barreto *et al.*, 2016), since market demand has now passed from fins to meat, which is sold as tuna. This can be seen clearly in the current case, since most fish substitutions were made with chondrichthyan species, mainly with sharks of the Triakidae family. Both the narrownose smooth-hound and tope shark were used to replace several species, such as the yellowfin tuna, stripped weakfish *Cynoscion guatucupa*, pink cusk-eel and Brazilian flathead *Percophis brasiliensis*. In addition, these sharks were the ones that received the greatest number of unofficial common names. Indeed, according to the International Red List of Endangered Species (IUCN, 2025), the tope shark, the narrownose smooth-hound and the spotback skate are classified as "critically endangered" (Finucci *et al.*, 2020; Pollom *et al.*, 2020; Walker *et al.*, 2020), and their population trends continue decreasing. Finally, the elephant fish is classified as "vulnerable", and its population trend is likewise declining.

Existing conservation measures in Argentina comprise closed areas and marine protected areas, a maximum allowable catch established annually by the Argentine fishing authorities, and a ban on landing sharks over 1.6 metres long, for commercial fishing vessels (Subsecretaría de Pesca y Acuicultura, 2020). However, much remains to be done to improve the effectiveness of these measures. Closed areas and marine protected areas do not systematically cover the different chondrichthyan assemblages found off the Argentine coast (Sabadin, 2019), and maximum allowable catch limits and the ban on landing large sharks are poorly enforced throughout the country. Consequently, approximately 47 percent of the chondrichthyan fauna of Argentina is considered to be at some of level of threat of extinction (vulnerable, endangered or critically endangered). Amendments to existing legislation should, at a minimum, include a requirement for the declaration on product labels of a designated "acceptable trade name" as well as the scientific name of the fish species being traded. An effort should be made to create a monitoring programme at the national level, to lead an intense focus on seafood certification. The government will also need to address the adequacy of the current regulations and monitoring processes. Fraudulent acts should be penalized according to the amount of mislabelling detected, as has been implemented in Brazil (Carvalho *et al.*, 2017). It is expected that this would greatly reduce the incidence of market substitution (Cline, 2012). If greater market transparency can be achieved, then public confidence in the Argentine seafood supply chain could be restored, and all efforts could be refocused on conserving ocean fish stocks.

Case study 4. Genetic-based identification of seafood mislabelling in restaurants, grocers and processing plants in Los Angeles, California

INTRODUCTION

Genetic-based DNA barcoding is a well-vetted approach that has been used to detect seafood mislabelling and fraud for over two decades (Marko *et al.*, 2004). The widespread adoption of DNA barcoding as a forensic tool has benefited from technological advances, which have increased accessibility and decreased sequencing costs (D. Willette *et al.*, 2014). Trust in the objectivity and reliability of molecular genetics has also grown, bolstered in part by the public's acceptance of at-home DNA test kits that enable individuals to learn about their genetic makeup, genealogy and biomedical health risks (Janzen *et al.*, 2005; Phillips *et al.*, 2018). In DNA-barcoding methods, a highly conserved region of the mitochondrial COI gene is used as a diagnostic tool to infer the closest species-level identification of a tissue sample by cross-referencing results with global, open-access genetic databases, including the Tree

of Life and Barcode of Life projects (Janzen *et al.*, 2005). These advances have enabled researchers and practitioners to begin addressing the harms of seafood fraud, including the overharvesting of marine fisheries (Pauly *et al.*, 2005) and the entry into the market of fish known to be hazardous to human health (Cohen *et al.*, 2009).

THE CASE IN THE LITERATURE

DNA-barcoding investigations of seafood fraud have been conducted in numerous countries. Such studies are often focused on the final point of the supply chain (for instance, restaurants) and are frequently limited to a single sampling event or period (Donlan and Luque, 2019). For example, studies have examined seafood mislabelling in Los Angeles, California (Khaksar *et al.*, 2015; K. Warner *et al.*, 2012), reporting single-year mislabelling rates of 16 percent to 55 percent in restaurants. DNA-barcoding studies, including those that span multiple years and levels of the supply chain, can help examine the effectiveness of measures for quality control, food-safety and truth-in-labelling regulations (Nehal *et al.*, 2021). Building upon the aforementioned studies in Los Angeles Willette *et al.* (2017, 2021) designed and conducted multiyear studies to track seafood mislabelling over time at the restaurant, grocer and processing-plant levels. These two studies used similar sample preservation, laboratory and bioinformatics protocols to permit comparison, although Willette *et al.* (2021) targeted ecolabelled seafood products. Willette *et al*. (2017) sampled 323 fish samples sold under nine common fish names from 26 sushi restaurants between 2012 and 2015, while Willette *et al.* (2021) sampled 123 fish from two processing plants and 149 fish from 13 grocers sold under 12 common fish names between 2017 and 2019. Notably, some, but not all, the same types of fish were targeted in both studies, due to the availability of consistently sold fish types. Combined findings from these two studies reveal a pattern of increased rates of seafood mislabelling through the supply chain. Seafood processing plants had the lowest mislabelling rate (4 percent), followed by grocers (11 percent), and sushi restaurants (47 percent). In both studies, mislabelling instances were not homogeneous across species. In sushi restaurants, fish sold as tuna, albacore (*Thunnus alalunga*) and salmon had a low mislabelling rate (<10 percent); whereas red snapper and halibut were mislabelled 100 percent of the time. In grocers, king salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*) and halibut were never found to be mislabelled, whereas black cod (*Anoplopoma fimbria*) and California halibut (*Paralichthys californicus*) were mislabelled 100 percent of the time. Mislabelling in processing plants was limited to a single fish type – Pacific halibut (*Hippoglossus stenolepis*) at 13 percent, with no other instances of mislabelling. In both studies, mislabelling rates were not found to differ statistically across sampling years. In Willette *et al.* (2021), the sampling years of 2018 and 2019 occurred after the implementation of the United States Seafood Import Monitoring Program (16 US Code § 1885), yet only Atlantic cod and albacore tuna were targeted species included in this federal reporting initiative, both with low to no instances of mislabelling in the study. In summary, these studies found that seafood mislabelling rates decreased moving back through the supply chain, yet did not fluctuate statistically between years, and that most instances of mislabelling are concentrated on a limited number of fish types.

SCALE AND GLOBAL INCIDENCE OF THE CASE

Incidents of seafood mislabelling in restaurants and grocers have been detected using DNA barcoding in at least 35 countries around the world (Willette *et al*., 2025). Such incidents in processing plants are less common (Shehata *et al*. 2019, Willette *et al*., 2021).

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

Seafood mislabelling and fraud cause multiple harms to consumers, society and the environment. Seafood mislabelling is a public-health concern as it may result in allergy or toxin exposure to consumers when products are inaccurately labelled as a different species. Mislabelling may also result in economic costs to consumers who do not receive the product they paid for and expected to receive, and it may deceive consumers about their dietary choices related to religious or environmental aspects (Giusti *et al.*, 2024).

TOOLS TO PREVENT THE ISSUE

DNA barcoding is a potent and frequently applied molecular-genetics tool used to investigate and validate the taxonomic identification of seafood, leveraging the highly accurate, low error rate of the Sanger sequencing method that has been used in studies from over 35 countries (Willette *et al.*, 2025). Further, the emergence of DNA metabarcoding (Giusti *et al.*, 2024) opens the possibility of scaling up molecular-based DNA monitoring of multiple seafood products simultaneously and of mixed-species products. Importantly, DNA barcoding and DNA metabarcoding are not substitutes for other surveillance tools, yet they are complementary techniques that are increasingly accessible around the world to build greater confidence in the accuracy of seafood labelling across the seafood supply chain.

CONCLUSIONS AND RECOMMENDATIONS

Detection of seafood mislabelling at multiple points in the supply chain indicates that addressing this challenge will require complementary action throughout the supply chain. Recommended actions include strengthening federal and state policies on product labelling, including consistent requirements for common and scientific names, as well as declarations of country of origin and method of capture. Such policies should be harmonized between state and federal levels, and where possible, harmonized with international regulations. Second, regular and mandatory DNA-based species-identification testing should be implemented to complement existing visual inspection and paper traceback systems.

Case study 5. Developing local partnerships to reduce seafood mislabelling – the Los Angeles Seafood Monitoring Project

This case study has a slightly different format but has been included as it demonstrates the value of combining the various tools described in the previous case studies, along with media and public awareness efforts, for effective mitigation of seafood fraud.

INTRODUCTION

Scientific studies using molecular methods to detect seafood mislabelling often garner media attention immediately after their publication. This was true, for example, in Los Angeles, California, for three scientific seafood-mislabelling studies conducted in 2012, 2015 and 2017 (Khaksar *et al.*, 2015; Warner *et al.*, 2012; Willette *et al.*, 2017), as well as for a legal case regarding tuna fraud by a popular sandwich restaurant chain (*Amin v. Subway Restaurants., Inc.*, 2023) and for an investigative study conducted by a popular media outlet (Flax, 2017). In the latter two examples, the names of surveyed restaurants were publicly disclosed. This was not the case in the first three scientific studies. Unfortunately, these studies alone have done little to reduce instances of seafood mislabelling in Los Angeles.

THE CASE IN THE LITERATURE

There is evidence, however, that seafood mislabelling does decrease when media attention is paired with broader community engagement, promotion of seafood literacy among consumers, or improved enforcement action (Mariani *et al.* 2015; Naaum and Hanner, 2015; Warner *et al.*, 2019). The Los Angeles Seafood Monitoring Project is a collaboration among local academia, industry and government stakeholders with the overarching aim of deterring, preventing and eliminating seafood mislabelling across the Greater Los Angeles area (Willette *et al.*, 2018). Project partners seek to (a) clarify ambiguity in local and federal seafood-labelling requirements, (b) provide best practices for labelling conventions in compliance with local and federal policies, and (c) conduct ongoing blind sampling and DNA-barcode testing of seafood sold by industry partners to enable tracking of changes in mislabelling rates and patterns over time. The project posts its recommendations, actions and findings on a public website (www.losangelesseafoodproject.org), including aggregate and anonymized results of the ongoing DNA-barcode testing. Recent results demonstrate that these sustained and collaborative efforts have led to a threefold reduction in seafood mislabelling rates in Los Angeles over the 10-year study period, from an average of 47 percent (Willette *et al*. 2017) to an average of 16 percent (Willette *et al*. 2025). This trend reflects the cumulative impact of public-facing education, consistent DNA-based monitoring and improved compliance and communication among vendors.

A salient example of the project's efforts to clarify seafood-labelling ambiguity pertains to members of the genus *Seriola*, a group of fish frequently mislabelled (>90 percent) in Los Angeles, as inferred from DNA-based identification testing (Willette *et al.*, 2017). In accordance with the U.S. Food and Drug Administration Seafood List (US FDA CPG Sec. 540.750, 2020), the only acceptable market name that may be used for five of the six *Seriola* species is "amberjack", with the acceptable market names for the sixth species being "amberjack" or "yellowtail". These *Seriola* species differ in price and taste, and are traditionally sold under unique names in Japan, essential differences that are lost in the ambiguous labelling scheme and deny both biological reality and Japanese culture.

PUBLIC HEALTH ASPECTS

Furthermore, different *Seriola* species may present higher health concerns at certain times of the year due to seasonal patterns in toxins. For example, wild-caught longfin yellowtail (*S. rivoliana*) can harbour ciguatoxins, which may lead to paralysis or death (Perez-Arellano *et al*., 2005). Suggested revisions to the *Seriola* labelling scheme were submitted by the Los Angeles Seafood Monitoring Project (Willette *et al.*, 2018) and are reflected in the listing of common names on the FDA Seafood List (FDA Seafood List, 1993).

CONCLUSIONS AND RECOMMENDATIONS

The sustained impact of these recommendations, alongside outreach and DNA surveillance, has demonstrated that structural interventions, rather than one-time investigations, are key to long-term reduction of seafood mislabelling (Willette *et al*., 2025). Efforts such as these help to reduce mislabelling instances that result from guideline limitations, allowing regulators to focus on intentional seafood fraud.

Case study 6. DNA barcoding reveals mislabelling of seafood in European Union mass caterings

INTRODUCTION

In 2016, a review was conducted analysing scientific papers related to seafood-mislabelling incidents worldwide that used DNA barcoding to detect real seafood mislabelling (Pardo and Jiménez, 2020). This review revealed an overall misdescription rate of 30 percent and a significantly higher rate in mass-catering food services (restaurants and takeaways).

THE CASE IN THE LITERATURE

In the European Union, the vast majority of seafood-mislabelling studies have focused on the retail end of the supply chain – mainly supermarkets and fishmongers, while few studies in mass caterings have studied samples from the hotel, restaurant and catering sector (Pardo and Jiménez, 2020). The first large-scale attempt to study the rate of fish mislabelling in the hotel, restaurant and catering sector across the European Union was launched in 2015, where a total of 283 samples were analysed by DNA barcoding. The samples were collected in 180 mass-catering outlets in 23 European Union countries (Pardo *et al.*, 2018). This study tried to elucidate the real percentage of mislabelling in the European Union, since previous studies revealed highly variable degrees of mislabelling, covering a reduced number of European Union cities and, in many cases, a reduced variety of fish species. In 2015, a French study analysed 100 fish samples in Paris with a surprisingly low mislabelling rate – just 3 percent (Bénard-Capelle *et al.*, 2015) Similar results were revealed in Italy and in the United Kingdom after analysing 185 and 115 samples (respectively) obtained in sushi restaurants (Mariani *et al.*, 2015). However, a study carried out in Brussels analysed 280 fish dishes sold in commercial restaurants, canteens and sushi bars and showed an overall 31.1 percent of mislabelled samples (Christiansen *et al.*, 2018). Finally, two species-specific studies conducted with tuna in Spain and Germany revealed higher mislabelling percentages: from 50 percent in Spain to 83 percent in Germany (Gordoa *et al.*, 2017; Kappel and Schröder, 2016). These discrepancies were the starting point of the largest study ever made, covering 23 European Union countries (Pardo *et al.*, 2018). The study concluded that 31 percent of the shops studied sold mislabelled seafood. Remarkable differences between countries were observed, with the highest mislabelling rate (50 percent) found in Finland, Germany, Iceland and Spain. However, the study recommended that specific national surveys be conducted to confirm their results. So far, a national survey was conducted in Spain, where 313 samples were collected in 204 mass caterers and analysed by DNA barcoding. The results showed that 50 percent of the establishments sold mislabelled seafood (Pardo and Jiménez, 2020). In addition, a recent study detected a mislabelling rate of 7.5 percent in the Belgian supply chain after analysing 53 samples sold as Atlantic cod and sole products in food catering (Deconinck *et al.*, 2020).

SCALE AND GLOBAL INCIDENCE OF THE CASE

This report, which focuses on fish fraud in Europe, presents findings similar to those reported in the United States and Argentina, as illustrated by two case studies. This reveals the global scale of fish mislabelling in the hotel, restaurant and catering sector.

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

The scientific community has pointed out the extremely hazardous risks that fish mislabelling may pose for public health. These risks include (i) the substitution of butterfish (*Peprilus triacanthus*), in sushi restaurants, with escolar (*Lepidocybium flavobrunneum*) – an oilfish with high levels of indigestible wax esters (Fariñas Cabrero *et al.*, 2015); (ii) the presence of poisonous species banned from the European

Union market (Armani *et al.*, 2015); (iii) the consumption of fish species (Nile perch and pangasius) contaminated with pollutants (Ferrantelli *et al.*, 2012; Filonzi *et al.*, 2010). Other implications include the commercialization of several highly endangered shark species, leading to significant negative impacts on ocean ecosystems (French and Wainwright, 2022).

TOOLS TO PREVENT THE ISSUE

The legislation in force in the European Union should be improved to include mandatory information (commercial designation and scientific name) in the labelling of processed products, including the fish we consume at restaurants. Strong food-control management programmes and enforcement through inspection, monitoring and control must be implemented by governments and the food industry, including the introduction of voluntary control systems.

CONCLUSIONS AND RECOMMENDATIONS

The detection of seafood mislabelling at multiple points in the supply chain indicates that addressing this challenge will require complementary actions throughout the supply chain. Recommended actions include strengthening European Union policies regarding product labelling, including consistent requirements to include the common and scientific names of products, country of origin and method of capture.

Case study 7. Tropical tuna misidentification in the canning industry

INTRODUCTION

Tuna is one of the most important fish species commercialized worldwide. In recent years, the canning industry has experienced a processing revolution: most canned products produced in the European Union use imported, frozen, skinned tuna fillets or loins from a variety of countries. These fillets and loins offer tremendous advantages in terms of productivity and yield, yet on occasion, in view of the difficulty of visually distinguishing between species, this process leads to species substitution. One interesting case is the distinction between tropical juvenile yellowfin tuna and bigeye tuna, which is challenging because these two species look very similar in their juvenile stages and are often caught together, along with other tropical tuna species, mainly skipjack tuna (*Katsuwonus pelamis*). Tropical tuna fishery catches generally include two main target species (yellowfin and skipjack tuna), but also a significant percentage of bigeye tuna, accompanied in different proportions by other secondary species. During the landing, a sorting of the target species is carried out by the crew according to commercial categories, more linked to the size of the individuals than to their species. A mixture of skipjack, yellowfin and bigeye juveniles in variable proportions are sent to processing plants, where tuna loins are prepared and provided to the European Union canning industry. Consequently, a lack of traceability arises from this complex supply chain – from overseas vessels, to processing plants, to the final canned products offered by retailers in the European Union.

THE CASE IN THE LITERATURE

These discrepancies in species composition estimates from tropical tuna landings were first detected in the mid-1980s by the Tropical Tuna Working Group of the International Commission for the Conservation of Atlantic Tunas, which focuses on juveniles. However, only one recent scientific paper has confirmed these early warnings (Carreiro *et al.*, 2023).

This case study focuses on tuna landings delivered to a processing plant in Cabo Verde, which primarily supplies the European Union. Using DNA barcoding, researchers identified taxonomic misassignments in 33 percent of the individuals among the three target tuna species: bigeye, yellowfin and skipjack. The results indicate that the mislabeling originated at the landing stage, generating negative ripple effects throughout the canning industry. Several studies have documented the substitution of yellowfin with bigeye and skipjack; skipjack with bigeye and yellowfin; and even the presence of mixed species within individual tuna cans. Sotelo *et al.* (2018) found a 7.8 percent mislabelling rate for canned tuna in European Union products. Servusova and Piskata (2021) also analysed canned tuna and found that 19.2 percent of skipjack and 24.4 percent of yellowfin cans were mislabelled, and one can was identified as a mix of yellowfin and skipjack. Bojolly *et al.* (2017) detected the presence of different species in yellowfin tuna cans, concluding that the mislabelling occurs during the production in the tuna canning industry (Bojolly *et al.*, 2017; Klapper *et al.*, 2023; Pardo *et al.*, 2018; Servusova and Piskata, 2021; Sotelo *et al.*, 2018).

SCALE AND GLOBAL INCIDENCE OF THE CASE

Tropical tuna fisheries supply the global canning industry. As such, this case has worldwide implications.

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

The main implication of this case study is from a conservation perspective. Bigeye tuna has been internationally assessed as vulnerable, while yellowfin and skipjack tuna populations have been evaluated as non-threatened. The mislabelling of tropical tuna species could hide underestimations in the assessment of bigeye tuna viability in the near future.

On the other hand, from a toxicological perspective, skipjack tuna has some of the lowest heavy-metal concentrations (including mercury). The mislabelling of other tuna species as skipjack may obscure the patterns of transference of these toxic substances to humans (Carreiro *et al*., 2023).

TOOLS TO PREVENT THE ISSUES

In this context, the sample size that a crew would have to manage onboard to correctly estimate the percentages of each tuna species would exceed 1 000 fish. This is not viable due to the difficulty in identifying correctly small-sized bigeye and yellowfin onboard, the urgency of transferring the catch to the wells as soon as possible and the requirement for qualified human resources. For this reason, DNA barcoding is the best solution to solve these analytical limitations, but the use of emerging methodologies, such as NGS and isothermal amplification, should also be considered.

CONCLUSIONS AND RECOMMENDATIONS

The mislabeling of tropical tuna species represents a critical challenge for the global canning industry. The increasing reliance on imported frozen loins and fillets, combined with the visual similarity of juvenile yellowfin and bigeye tuna, creates a high risk of species substitution early in the supply chain. DNA barcoding studies confirm that these errors originate at the landing stage and propagate through processing and retail, undermining traceability and product integrity. The implications are significant. From a conservation standpoint, mislabeling can mask the true exploitation of vulnerable species such as bigeye tuna, compromising stock assessments and management strategies. From a public health perspective, inaccurate species identification may distort monitoring of heavy metal exposure, particularly when species with lower contaminant levels, such as skipjack, are substituted. Operational constraints make accurate onboard sorting impractical, highlighting the need for molecular tools. DNA barcoding offers

a reliable solution, and emerging technologies such as next-generation sequencing and isothermal amplification should be considered to improve efficiency and scalability. Strengthening traceability across the tropical tuna supply chain is essential to protect marine resources, ensure consumer safety, and maintain the credibility of the global tuna market.

Case study 8. Substitution of frozen-thawed fish for fresh fish

INTRODUCTION

Fish traded globally often have long and complex supply chains, with significant distances from the places where they are captured or harvested to where they are consumed. Because fish are highly perishable, they have traditionally been preserved using chilling (in the case of fresh products) and freezing methods. In the fish industry, the term "fresh" denotes that the fish has never been frozen along the entire supply chain, from its capture until its commercialization. However, for long supply chains, even though numerous preservation methods have been used in the industry, freezing is the most frequently used technology (Sotelo *et al*., 2018; Verrez-Bagnis *et al*., 2017). This process converts the available water into ice crystals (Gram and Huss, 1996), but it can affect the fish's organoleptic characteristics. Although it is feasible to obtain high-quality frozen products, fresh fish is deemed superior, as freezing may result in changes in colour, texture and water-holding capacity, as well as structural damage caused by intracellular/extracellular ice-crystal growth (Dawson, Al-Jeddawi and Remington, 2018). The preference of consumers for fresh fish is based on sensory characterizations, as apparent alterations in the flavour, odour, consistency and colour of flesh can occur during freezing, frozen storage and thawing (Claret *et al*., 2012). This makes fresh fish more expensive than frozen fish, which creates opportunity for fraud in the form of substituting fresh fish with frozen-thawed fish to generate higher profits. In some places, however, such as the European Union, freezing fish is mandatory for fishery products intended to be consumed raw (EU, 2011). In any case, according to European Union regulations, frozen-thawed fish must be labelled as defrosted or previously frozen and must not be refrozen (FAO, 2010). Otherwise, it is considered fraudulent mislabelling.

There is limited information on the occurrence of this type of mislabelling. The few mentions found in official reports are based in the European Union. No figures from other regions of the world were found. However, numerous studies on other forms of fish mislabelling have been published worldwide and, in 2012 and 2014, the European Union adopted additional provisions requiring a more stringent system for traceability and labelling of fish products, from catch or harvest through retail. According to these provisions, fish labels should include information such as the commercial and scientific name, fishing-gear category, production method, catch or production area, "best before"/"use by" date, storage conditions, net weight, information about allergens and whether the product is fresh, frozen or previously frozen (Warner *et al*., 2016). Still, concerns regarding the substitution of fresh fish products with frozen-thawed products is increasing, not only within the European Union but worldwide.

THE CASE IN THE LITERATURE

Accurate differentiation between fresh and frozen-thawed fish is challenging because their chemical and physical characteristics are very similar (Karoui *et al*., 2006). The absence of a gold standard for the determination of whether a fish product has been previously frozen (EFSA, 2021) makes fish highly vulnerable to mislabelling. Thus, official reports of this type of fraud are scarce, and those that are available rely on qualitative evaluations such as muscle consistency, eye opacity, etc. (Bozzetta *et al*., 2012). This also has an impact in the absence of figures regarding the incidence of this type of fraud.

In order to state the case in the literature, a search was conducted in Google Scholar, exploring literature from 1980 to the present. Although official figures on the incidence of this type of fraud were not found, the extensive amount of scientific literature focusing on the study of different techniques and methodologies to detect this type of fraud illustrates that this type of mislabelling is an important concern. For this case study, 127 scientific papers focusing on such methodologies were reviewed, concentrating on the methods to determine whether a fish product has been previously frozen and thawed. Traditionally, analytical methods have been used. However, these methods are not suitable for real-time detection because they require specific laboratory equipment, are destructive and may be time consuming. The methods can be divided into different categories, such as biochemical, morphological, organoleptic, microbiological or the combination of several of these methods (Table 5). Among the specific analytical methods available, the most used are enzymatic, histological, measurement of volatile composition and microbiological growth associated with the thawing processes (Hassoun *et al*., 2020a; Sotelo *et al*., 2018; Verrez-Bagnis *et al*., 2017). Also, non-destructive methods (Table 6), especially those based on spectroscopy, are becoming more prevalent in recent years. These methods are suitable for use *in situ* because they do not require sample preparation or reagents and provide information in real time. These methods are always coupled with chemometrics or data analysis and allow for the rapid and easy testing of a high number of samples (Nieto Ortega, 2023). Spectroscopic techniques are the most used, namely vibrational (NIR, mid-infrared and Raman), fluorescence or absorption UV-Vis, and NMR spectroscopy. Also, spectroscopic techniques based on imaging, such as hyperspectral imaging (HIS) are being used as alternatives to traditional methods (Ghidini *et al*., 2019b). Indeed, according to the review performed by Ghidini *et al*. (2019b) on these types of techniques for the detection of fish mislabelling, 32 percent of the techniques focused on fresh/thawed substitution, 26 percent focused on the production method detection, 23 percent on species substitution and 19 percent on geographical origin.

TABLE 5
Review of destructive methods used for fresh/thawed state identification

Method category	Method group	Identification criteria	Fish species	Reference
Biochemical	Enzymatic methods	Mitochondrial enzymes	Gilthead sea-bream (*Sparus aurata*)	Bouchendhomme *et al.*, 2023
			Yellowfin tuna (*Thunnus albacares*)	Bernardi *et al.*, 2019
			Sole (*Solea solea*), sea-bream (*Pagellus centrodontus*), hake (*Merluccius merluccius*), gilt headed bream (*Sparus aurata*), seabass (*Dicentrarchus labrax*), salmon (*Salmo salar*), prawn (*Penaeus japonicus*) and Norwegian lobster (*Nephrops norvegicus*)	Fernández-Segovia *et al.*, 2012
			Crawfish (*Procambarus clarkii*) and trout (*Salmo gairdneri*)	Hoz *et al.*, 1992
		Lysosomal enzymes	Anchovies (*Engraulis encrasicolus*), sardines (*Sardina pilchardus*), horse mackerels (*Trachurus trachurus*) and chub mackerels (*Scomber japonicus colias*)	Alberio *et al.*, 2014
			Salmon fish species (*Oncorhynchus keta* and *Salmo salar*)	Akhtar *et al.*, 2013
		Cytosolic enzyme	Sea-bream (*Sparus aurata*)	Diop *et al.*, 2016
	Electrophoresis	Protein markers	Seabass (*Dicentrarchus labrax*)	Marlard *et al.*, 2019
	Haematological	Haematocrit value	Carp	Yoshioka, 1985
		Haemolysis of red blood cells	Anglerfish (*Lophius* spp.), cod (*Gadus morhua*), salmon (*Salmo salar*), swordfish (*Xyphius gladius*)	Civera *et al.*, 1996
	DNA degradation	Commet assay	Salmon (*Salmo salar*)	Julie Le, 2013
	Measurement of volatile composition	Gas chromatography-mass spectrometry	Cod (*Gadus morhua*), salmon (*Salmo salar*), European Union seabass (*Dicentrarchus labrax*), gilthead sea-bream (*Sparus aurata*)	Leduc *et al.*, 2012
			Gilthead sea-bream (*Sparus aurata*)	Iglesias *et al.*, 2009
		Ambient mass spectrometry (AMS)	European Union seabass (*Dicentrarchus labrax*)	Massaro *et al.*, 2021
		Electronic nose	Trout	Di Natale *et al.*, 2000
	Metabolomics	High resolution mass spectrometry (LC-HRMS)	European Union seabass (Dicentrarchus labrax)	Stella *et al.*, 2022
			Salmon (*Salmo salar*), bullet tuna (*Auxis rochei*)	Chiesa *et al.*, 2020
Morphological	Histological	Recognition of structural changes at microscopic level	Salmon	Pezzolato *et al.*, 2020
			European Union hake (*Merluccius merluccius*)	Tinacci *et al.*, 2018a
			Anchovy (*Engraulis encrasicolus*)	Bozzetta *et al.*, 2012; Pezzolato *et al.*, 2020
			Gilthead (*Sparus auratus*), red mullet (*Mullus barbatus*), and swordfish (*Xiphias gladius*)	Bozzetta *et al.*, 2012; Pezzolato *et al.*, 2020
	Eye-lens analysis	Observation of the medulla of the crystalline	Cod (*Gadus callarias*) and haddock (*G. aeglefinus*)	Love, 1956
			Carp (*Cyprinus carpio*), common horse mackerel (*Trachurus japonicus*), eel (*Anguilla japonica*), flat fish (*Limanda herzensteini*), Pacific mackerel (*Pneumatophorus japonicus japonicus*), porgy (*Chrysophrys major*), yellowtail (*Seriola quinqueradiata*)	Yoshioka and Kitamikado, 1983
			Mackerel (*Scomber scombrus*), plaice (*Pleuronectes platessa),* whiting (*Merlangus merlangus*)	Duflos *et al.*, 2002

TABLE 5
Review of destructive methods used for fresh/thawed state identification (continued)

Method category	Method group	Identification criteria	Fish species	Reference
Organoleptic	Consumer panel	Sensorial characteristics: taste and flavour, fattiness, smooth mouth feel, retro-nasal aroma, redness of meat, overall appearance, texture, springy palatability, wateriness	Sashimi from Pacific saury (*Cololabis saira*)	Watanabe *et al*., 2020
Microbiological	Microbiological activity	Measurement of total viable counts, counts of Enterobacteriaceae and psychrotrophic bacteria	Rainbow trout (*Oncorhynchus mykiss*)	Popelka *et al*., 2014
Combination of techniques		Protein concentration, β-D-glucosidase activity, nucleotides and related compounds (NRCs) concentration, and free calcium	Seabass (*Dicentrarchus labrax*)	Marlard *et al*., 2019
		Enzymatic assays, chemical composition, expressible drip, total volatile nitrogen (TVN), microbiological analyses, histological examinations	Rainbow trout (*Oncorhynchus mykiss*)	Popelka *et al*., 2014

TABLE 6
Non-destructive methods used for fresh/thawed state identification

Method category	Method group	Basis	Fish species	Chemometrics/data analysis	Identification criteria	Reference
Spectroscopy	Front-face fluorescence	Absorption, emission, fluorescence	Whiting (*Merlangius merlangus*)	PCA + FDA	Nicotinamide adenine dinucleotide (NADH)	Karoui *et al.*, 2006
			Seabass (*Dicentrarchus labrax*)	CCA + FDA + CCSWA	Color, textural and chemical parameters	Karoui *et al.*, 2017
	Ultraviolet		Tuna (*Thunnus obesus*)	PLS-DA	Water, fat in the fish muscle	Nieto-Ortega, *et al.*, 2022
			Tuna (*Thunnus* thynnus)	PLS-DA	Structural alternations of myoglobin and its oxidative products	Reis *et al.*, 2017
			Atlantic salmon (*Salmo salar L.*)			Kimiya *et al.*, 2013
	Visible		Gilthead sea-bream (*Sparus aurata*), red mullet (*Mullus barbatus*), sole (*Solea vulgaris*), swordfish (*Xiphias gladius L.*)	PLSR and PCA	Changes in heme proteins	Ottavian *et al.*, 2013
			Red sea-bream (*Pagrus major)*	PLS-DA and orthogonal decomposition + PLS-DA	Water absorbance	Uddin *et al.*, 2005
				LDA and SIMCA		
	Near infrared	Absorption				
	Mid-infrared		Red mullet (*Mullus surmuletus*), Atlantic mullet	LDA and SIMCA	Water, aliphatic group, amines	Alamprese and Casiraghi, 2015
			Whiting (*Merlangius merlangus*)	PCA + FDA	Water content and fatty acids	Karoui *et al.*, 2017
	Raman	Vibration	Horse mackerel (*Trachurus trachurus*), European Union anchovy (Engraulis encrasicolus), Red mullet (Mullus surmuletus), bluefish (Pomatamus saltatrix), Atlantic salmon (Salmo salar) and Flying gurnard (Trigla lucerna)	PCA	Lipid structure involving CH groups	Velio□lu *et al.*, 2015
	Radio wave	Nuclear magnetic resonance (NMR) and Nuclear magnetic resonance imaging (MRI)	Salmon (*Salmo salar*)	PCA + ANOVA	Measurement of aspartate in frozen-thawed samples	Shumilina *et al.*, 2020
			Rainbow trout (*Salmo gairdneri*)	-	Tissue water	Nott *et al.*, 1999

TABLE 6
Non-destructive methods used for fresh/thawed state identification (continued)

Method category	Method group	Basis	Fish species	Chemometrics/data analysis	Identification criteria	Reference
			Cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*)	-	Connective-tissue breakdown	Howell *et al.*, 1996
	Impedance	Electrical	Sea-bream (*Sparus aurata*)	ANOVA + PCA	IMP, TBA index, WHC and textural parameter	Fuentes *et al.*, 2013
			Salmon (*Salmo salar*)	ANOVA and PCA + DA	Water-holding capacity	Fernández-Segovia *et al.*, 2012
	Hyperspectral imaging	Absorption/emission/ scattering	Red snapper (*Lutjanus campechanus*)	24 classifiers as: decision trees, discriminant analysis, Naive Bayes classifiers, support vector machines, k-nearest neighbour classifiers, and ensemble classifiers	Water content	Qin *et al.*, 2020
			Halibut (*Psetta maxima*)	LS-SVM classifiers	Water content, texture and colour	Zhu *et al.*, 2013
			Atlantic salmon (*Salmo salar L.*)	PLSR and PCA	Changes in heme proteins	Kimiya *et al.*, 2013
Electric	Time-domain reflectometry	Dielectrical	Tuna (*Thunnus obesus*)	PLS-DA	Dielectric properties due to water, fat in the fish muscle	Nieto-Ortega *et al.*, 2022
	Bioelectrical impedance analysis (BIA)	Electrical	Tuna (*Thunnus obesus*)	PLS-DA	Muscle, protein and cell membranes	Nieto-Ortega *et al.*, 2022
			Albacore tuna (*Thunnus alalunga*)	PCA, DA and ANOVA	Cell membranes	Cox, 2015
			Seabass *(Dicentrarchus labrax)*	PCA	Dielectrical and electrical properties	Vidaček *et al.*, 2008

Notes: DA: discriminant analysis, PCA: principal component analysis, CCA: canonical correlation analysis, FDA: factorial discriminant analysis, CCSWA: multiblock analysis method based on common components and specific weights analysis, PLS-DA: partial least-squares discriminant analysis, PLSR: partial least-squares regression, LS-SVM: least-squares support vector machine, LDA: linear discriminant analysis, SIMCA: soft independent modeling of class analogy, ANOVA: analysis of variance.

SCALE AND GLOBAL INCIDENCE OF THE CASE

Substitution of frozen-thawed fish for fresh fish is a common type of fraud occurring worldwide. Although there are regulations in the European Union (EU Regulation No. 1169/2011 and EU Regulation No. 1379/2013) that require the declaration of whether the fish is fresh, frozen, or has been previously frozen and the preservation treatment used, in some other regions and countries no regulations exist. Most countries have no government agency responsible for regulating fish fraud, which further complicates the ability to obtain reliable data about the problem at the country level. Furthermore, there is no official methodology nor standards at national and international levels for detecting this type of fraud, and most detection is based on qualitative evaluation, which is subjective, or on the use of analytical methods, which are time consuming and require qualified personnel. Thus, official control reports are scarce. However, it is known that the occurrence of this type of substitution is a concern, and numerous attempts are being made to develop rapid methods that can provide information in real time to detect previously frozen products that are mislabelled.

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

The substitution of frozen-thawed for fresh fish products has several implications. Apart from those related to economic issues and the loss of consumer trust, there are other implications related to health and food quality. Since fish is an extremely perishable food product, enzymatic and microbiological activity increase dramatically after death. Freezing slows down these processes. However, improper storage and handling could lead to increased bacterial growth, especially that of *Pseudomonas, Lactobacillus, Proteus* and *Shewanella putrefaciens* (Noor Uddin *et al.*, 2013), in addition to the growth of other bacteria that cause unpleasant odours due to the degradation of amino acids that convert into biogenic amines, sulphides, organic acids and other compounds (Stratev *et al.*, 2015). Furthermore, thawing is a slow, non-uniform process, with some regions of the fish being exposed to higher levels of microbial growth due to the temperature increase (Akhtar *et al.*, 2013), moisture and available nutrients. The optimal freezing temperature is -18 ºC. When the process is not controlled, some psychrotrophic microorganisms can still grow, for instance at temperatures above -10 ºC (Opoku-Nkoom, 2015). Quality may also be affected if the freezing/thawing process is done incorrectly, since freezing rate is a critical parameter for the size and shape of intracellular and extracellular ice crystals (Alizadeh *et al.*, 2007). When ice crystals are irregular they may cause cellular damage. Furthermore, if they are formed in extracellular spaces, they may cause diffusion of water out of the cells, causing tissue dehydration. Also, the freezing process increases the concentration of enzymes and salt, causing protein denaturation and drip loss, which affect fish texture (Chevalier *et al.*, 2000). A consumer risk could also arise when frozen-thawed fish is mislabelled or misrepresented as fresh. Consumers may unknowingly refreeze the product, believing it has not been previously frozen. This second freezing can significantly compromise both safety and quality (Hu and Xie, 2021). Repeated freeze/thaw cycles can exacerbate microbial proliferation, especially if the product has already undergone partial spoilage (Ong and Borris, 2025; Elbarbary *et al.*, 2023). Additionally, the structural integrity of the fish deteriorates further, increasing the risk of texture degradation, nutrient loss and the formation of harmful compounds. This misperception can lead to unsafe consumption practices and increased exposure to foodborne pathogens (Du *et al.*, 2023)

TOOLS TO PREVENT THE ISSUE

Of primary importance in preventing this issue is the establishment of regulations. Though specific regulations already exist in the European Union (Commission Regulation [EU] No 1276/2011), no relevant legislation was found for other regions and countries of the world. In addition, a full-chain traceability framework that determines all the processes that the fish has undergone is needed. For this purpose, collaboration between different institutions and the existence of regulatory entities to prevent fish fraud is of utmost importance. The boom of Industry 4.0-enabling technologies presents an unprecedented opportunity to trace the entire supply chain. Some tools, such as electronic labels, QR codes and barcodes, could be used to have real-time information about the processes of the fish product. As stated before, reliable methods to determine whether a fish product has previously been frozen and thawed are needed. The absence of a gold standard also makes inspection difficult. Traditional methods require expensive equipment, are time consuming and require specialized personnel. On the other hand, non-destructive technologies – especially cost-effective, portable versions, open the door to establishing a standardized methodology in the industry, but standards must be defined. In addition, such technologies must be continuously calibrated through chemometrics and machine learning. Yet, these technologies would offer a rapid response that could be useful in quality control at several stages of the value chain (EFSA BIOHAZ Panel, 2021).

CONCLUSIONS AND RECOMMENDATIONS

Fish products are vulnerable to mislabelling fraud, especially the substitution of frozen-thawed fish for fresh fish, which is associated with the increase in worldwide consumption of fish and the higher prices associated with fresh fish. This form of mislabelling can lead to public-health problems, a decrease in the quality of the products, loss of consumer trust and economic losses. The absence of regulations in different parts of the world, together with the absence of a gold standard to determine whether a fish product has been previously frozen, makes it very difficult to have a global picture of the incidence of this type of fraud. The fact that there are efforts in researching new, rapid tools to detect such fraud illustrates that this form of mislabelling is an important concern. In this sense, there is a need for consensus between the scientific community, public-health agencies and regulatory institutions to determine a methodology that allows for the detection of this type of fraud.

Case study 9. Misrepresentation of production method: the case of farmed versus wild-caught seafood

INTRODUCTION

The aquaculture industry has experienced significant growth over the past century, increasing from a 4 percent share of total fishery and aquaculture production in the 1950s to a 49 percent share in 2020 (FAO, 2022a). Of the 178 million tonnes of seafood produced globally in 2020, 90 million tonnes came from capture fisheries and 88 million tonnes were produced through aquaculture (FAO, 2022a). In contrast, capture-fishery production has stabilized over the past 30 years despite growing demand, and approximately 30 percent of fish stocks are considered to be overfished (Mangin *et al.*, 2021). The cost of aquaculture production has dropped over time and retail prices for farmed seafood tend to be lower than those of wild-caught seafood. In addition, aquaculture generally produces greater volumes of seafood that are available throughout the year (Stiles *et al.*, 2013). By helping meet the increased global demand for animal protein, aquaculture can alleviate some of the strain placed on overharvested wild seafood populations (Brayden *et al.*, 2018). However, aquaculture

production has its own set of concerns, including the use of antibiotics and pesticides, disease brought on by overcrowding, and negative effects on native stocks (Brayden *et al.*, 2018). Despite the increasing availability and relatively low price of farmed seafood, consumers tend to prefer wild seafood due to the perception that it is of higher quality (Muñoz-Colmenero *et al.*, 2017). While farmed seafood can also be a source of high-quality protein, consumers cite factors such as taste, health and nutrition in supporting their preference for wild seafood (Pulcini *et al.*, 2020; Verbeke *et al.*, 2007). Given the relatively high consumer demand and pricing for wild-caught seafood, there is an economic incentive associated with mislabelling farmed seafood as wild caught. Additionally, the similar appearance of many seafood species makes it difficult for consumers to recognize when seafood has been mislabelled (Silva *et al.*, 2021). The purpose of this case study is to provide examples of seafood known to be misrepresented based on production method, to discuss the public-health and environmental implications of this form of mislabelling, and to describe the current tools available for combating this type of fraud.

THE CASE IN THE LITERATURE

Some of the main species of seafood reported to be mislabelled on the basis of production method are salmon, seabass and shrimp (Table 7). Although several studies have been published describing the mislabelling of farmed seafood as wild caught, there is a general lack of research on this aspect of seafood fraud (Pardo *et al.*, 2016). Thus far, most reports of this type of fraud have focused on instances where a species known to be exclusively or almost exclusively farmed is mislabelled as being wild caught. In these cases, production-method mislabelling can be readily detected using standardized DNA-based tools for species identification. Other instances of production-method mislabelling (for instance, when a single species is both farmed and wild caught) require more complex analytical tools for detection and are therefore less extensively applied to examine fraud in the commercial marketplace.

TABLE 7
Examples of seafood reported to be mislabelled based on production method

True identity	Mislabelled as	Geographic regions	Detection method	References
Farmed (Atlantic) salmon or rainbow trout	Wild (Pacific) salmon	Canada, United States of America	DNA barcoding	Cline, 2012; Warner *et al.*, 2016; Warner *et al.*, 2019
Farmed salmon	Wild salmon	United States of America	Measurement of synthetic astaxanthin levels	Burros, 2005
Farmed salmon or trout	Wild salmon	Norway	Isotopic analysis and fatty-acid composition	Thomas et al., 2008
Farmed rainbow trout	Wild native brown trout	Spain	DNA barcoding	Muñoz-Colmenero *et al.*, 2017
Farmed, imported shrimp	Wild local shrimp	United States of America	DNA barcoding	Korzik *et al.*, 2020
Farmed, imported shrimp; farmed, imported sutchi	US wild shrimp; wild sole	United States of America	Federal Investigation	U.S. Department of Justice, 2011
Farmed European Union seabass	Wild European Union seabass	Italy	Multivariate analysis	Fasolato *et al.*, 2010

The substitution of farmed Atlantic salmon or rainbow trout for wild Pacific salmon is one of the more widely reported examples of production-method mislabelling (Table 7). The name Pacific salmon refers to a group of six closely related species, including chum (*O. keta*), coho (*O. kisutch*), Chinook (*O. tshawytscha*), pink (*O. gorbuscha*), sockeye (*O. nerka*) and cherry (*O. masou*) salmon (Cline, 2012). These species are all primarily wild caught, with limited farming of coho, sockeye and Chinook salmon (Cline, 2012; Warner *et al.*, 2016). On the other hand, Atlantic

salmon and rainbow trout are almost exclusively farmed species produced worldwide in countries and regions such as Chile, Europe and North America, with very limited wild harvest (NOAA Fisheries, 2022; FAO, 2025b; Tom and Olin, 2010; FAO, 2022b). Globally, farmed Atlantic salmon constitutes over 90 percent of the farmed salmon market and more than 50 percent of the total global salmon market (FAO, 2025b). Due to the relatively high price of wild-caught salmon, there is an economic incentive to mislabelling farmed salmon or trout as wild caught (Cline, 2012; Stiles *et al.*, 2013; Tom and Olin, 2010).

Several studies have reported mislabelling of farmed salmon or trout as wild salmon in the commercial marketplace (Cline, 2012; Warner *et al.*, 2013; Warner *et al.*, 2015; Hu *et al.*, 2018; Thomas *et al.*, 2008; Burros, 2005; Stiles *et al.*, 2013; Consumer Reports, 2006). In studies that have used DNA testing, it is assumed that samples identified as Atlantic salmon are farmed due to the extremely limited availability of wild-caught Atlantic salmon. (Less than 1 percent of commercially available Atlantic salmon are harvested in the wild [Tom and Olin, 2010]). For example, in a series of studies conducted in the United States, it was reported that 11 percent of samples labelled as various Pacific salmon species were instead identified as Atlantic salmon (Cline, 2012), while Warner *et al.* (2015) reported that 69 percent of samples labelled as wild salmon were actually identified as Atlantic salmon or rainbow trout. In a study using a combination of chemical analyses, multiple samples collected in Norwegian supermarkets labelled as "wild salmon" were instead determined to be farmed salmon or trout (Thomas *et al.*, 2008). Interestingly, research has suggested that the degree of salmon mislabelling is dependent on the time of year, with lower rates of mislabelling recorded during the salmon fishing season as compared to the off-season (Warner *et al.*, 2015, Warner *et al.*, 2013; Cline, 2012; Consumer Reports, 2006). This suggests that mislabelling may be driven by a reduction in the availability of fresh wild-caught salmon during the off-season (Cline, 2012). Another study reported the mislabelling of farmed rainbow trout (*Oncorhynchus mykiss*) as wild native brown trout (*Salmo trutta*) in multiple samples collected in Spain (Muñoz-Colmenero *et al.*, 2017).

SCALE AND GLOBAL INCIDENCE OF THE ISSUE

As indicated, the substitution of farmed for wild-caught seafood, especially farmed salmon and trout, has been documented globally. With aquaculture production providing an increasing proportion of seafood to the global population, farmed seafood has become more accessible and affordable. However, consumer preferences for wild seafood, combined with price differentials between farmed and wild seafood, have created strong incentives for mislabelling. As demonstrated in Table 1, salmon, shrimp and seabass have all been found to be misrepresented based on production method. The most widespread form of production-method mislabelling involves farmed salmon and trout being falsely represented as wild, with documented cases in Europe and North America. Instances of mislabelling of farmed shrimp as wild shrimp have been reported in the United States, while the substitution of farmed seabass for wild seabass was previously detected in Italy.

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

In addition to the economic deception associated with production-method misrepresentation, it also presents numerous public-health and environmental concerns. With regard to public health, some farmed species contain different nutritional profiles as compared to wild seafood (Szlinder-Richert *et al.*, 2011). Mislabelling of these species can impact consumers who are seeking specific seafood products based on nutritional benefits, such as omega 3 fatty-acid content (Naaum *et al.*, 2016). Furthermore, some farmed seafood may contain higher levels of environmental contaminants or antibiotic residues. It is important that these products are correctly

labelled so they can be properly screened for environmental contaminants and for any banned compounds (Naaum *et al.*, 2016). Mislabelling of the production method may result in contaminated products evading inspection protocols and erroneously entering the commercial marketplace. In this way, fraudsters can circumvent targeted inspection of farmed products and command higher prices by selling the products as "wild", thereby providing a double incentive to misrepresent the production method (Naaum *et al.*, 2016). For example, three owners of US seafood wholesalers were sentenced to prison for their roles in purchasing and selling mislabelled seafood, including farmed sutchi (*Pangasianodon hypophthalmus*) imported from Vietnam, mislabelled as wild-caught sole, as well as imported farmed shrimp mislabelled as wild-caught US shrimp (U.S. Department of Justice, 2011). By mislabelling sutchi as sole, the owners avoided paying close to USD 150 000 in anti-dumping duties associated with sutchi and other *Pangasius* spp. Furthermore, malachite green and Enrofloxacin were detected in several of the fish seized during the investigation. These compounds are prohibited in US foods but are known to be used in some fish-farming operations outside of the United States.

Misrepresentation of the production method also interferes with the ability of consumers to make informed purchasing decisions. Certification programmes meant to increase consumer awareness of sustainable seafood choices have increased with consumer demand for ecofriendly, natural and organic products (Gulbrandsen, 2009; Uchida *et al.*, 2014; Willette *et al.*, 2017). However, the effectiveness of these programmes is dependent on accurate labelling of production method, as well as species and provenance, combined with supply-chain traceability (Willette *et al.*, 2017). The mislabelling of a farmed species associated with unsustainable aquaculture practices as a wild-caught species from a sustainably managed fishery not only interferes with purchasing decisions, but also promotes sales of unsustainable seafood.

TOOLS TO PREVENT THE ISSUE

There are a variety of analytical tools available to detect production-method mislabelling. The mislabelling of a species that is almost exclusively farmed as "wild-caught" can be detected using DNA-based tools for species identification, such as DNA barcoding (Cline, 2012; Warner *et al.*, 2015; Warner *et al.*, 2013; Hu *et al.*, 2018; Muñoz-Colmenero *et al.*, 2017; Korzik *et al.*, 2020). However, in situations where the same species is both farmed and wild caught, alternative analytical tools are required for detection, such as liquid chromatography, gas chromatography/mass spectrometry (GC/MS), multi-element profiling, stable isotope analysis, proton nuclear magnetic resonance ([1]H NMR) spectroscopy, or near-infrared spectroscopy (NIRS) (Turujman *et al.*, 1997; Maestri *et al.*, 2018; Mannina *et al.*, 2008; Ottavian *et al.*, 2012; Arechavala-Lopez *et al.*, 2013). For example, liquid chromatography can be used to detect farmed salmon based on the presence of synthetic astaxanthin in the fish flesh (Turujman *et al.*, 1997). Stable isotopes have been used in several studies to differentiate farmed from wild seafood, including shrimp (Wang *et al.*, 2018) and salmon (Wang *et al.*, 2018). Fatty-acid analysis is a powerful method for differentiating farmed and wild seafood and has been widely researched in fish such as gilthead sea-bream (*Sparus aurata*) and European seabass (Arechavala-Lopez *et al.*, 2013) as well as salmon (Grazina *et al.*, 2020; Megdal *et al.*, 2009). More recently, another study used a combination of chemometrics and elemental fingerprinting to correctly identify the production method of wild and farmed gilthead sea-bream in the majority of test samples (Mamede *et al.*, 2022).

Oftentimes, a combination of chemical-based methods is used for improved accuracy (Chaguri *et al.*, 2017; Fasolato *et al.*, 2010; Thomas *et al.*, 2008). For example, numerous studies have used a combination of isotopic ratio analysis and multi-element profiling to differentiate farmed from wild seafood species (Li *et al.*, 2016), including

carp (*Cyprindae* family) (Liu *et al*., 2020), European seabass (Varrà *et al*., 2019) Asian seabass (Gopi *et al*., 2019), shrimp (Ortea and Gallardo, 2015) and salmon (Anderson *et al*., 2010). Other studies have shown that a combination of isotopic ratio analysis and fatty-acid composition can be used to differentiate between wild and farmed European seabass (Thomas *et al*., 2008; Bell *et al*., 2007) and salmon (Thomas *et al*., 2008). Farabegoli *et al*. (2018) conducted multivariate analysis to authenticate wild and farmed European seabass considering biometric traits, fatty-acid profile, elemental composition and isotopic abundance. They reported that fatty-acid profiles showed the most accurate results. An emerging method that examines the microbiome of seafood has shown promising results for tracing the geographical origin of seafood (Milan *et al*., 2019; Pimentel *et al*., 2017) and may prove to be an effective tool in differentiating between farmed and wild seafood (Ramírez and Romero, 2017). While analytical tools have an important role in detecting the misrepresentation of the production method, they should not be used as the sole means of preventing fraud. Rather, they should be used as part of a comprehensive food-fraud mitigation plan that includes supplier audits and rigorous supply-chain traceability (Lees and Reimann, 2021; Naaum and Hanner, 2015).

CONCLUSIONS AND RECOMMENDATIONS

Seafood is highly vulnerable to fraud related to the misrepresentation of the production method, in part due to the greater consumer demand and higher prices associated with wild-caught seafood. The mislabelling of farmed salmon or trout as a wild-caught product appears to be the most widely reported type of production-method misrepresentation, with limited instances of production-method mislabelling reported for other species (such as shrimp and European seabass). In addition to the economic consequences of production-method mislabelling, there are numerous public-health concerns due to differences in nutritional composition and contaminants of some farmed and wild seafood. Additionally, production-method misrepresentation interferes with consumer purchasing decisions and depletes the effectiveness of seafood-certification programmes meant to promote sustainability. Several analytical tools have been developed to differentiate between farmed and wild seafood, including DNA-based tests and chemical analyses. However, it has proven challenging to discriminate between wild and farmed populations of the same species, and in these cases multivariate analyses are often carried out using a combination of analytical methods and chemometrics. In addition to analytical tools, a comprehensive food-fraud mitigation plan involving rigorous supply-chain traceability is essential in combating production-method misrepresentation.

Case study 10. Geographical-origin mislabelling

INTRODUCTION

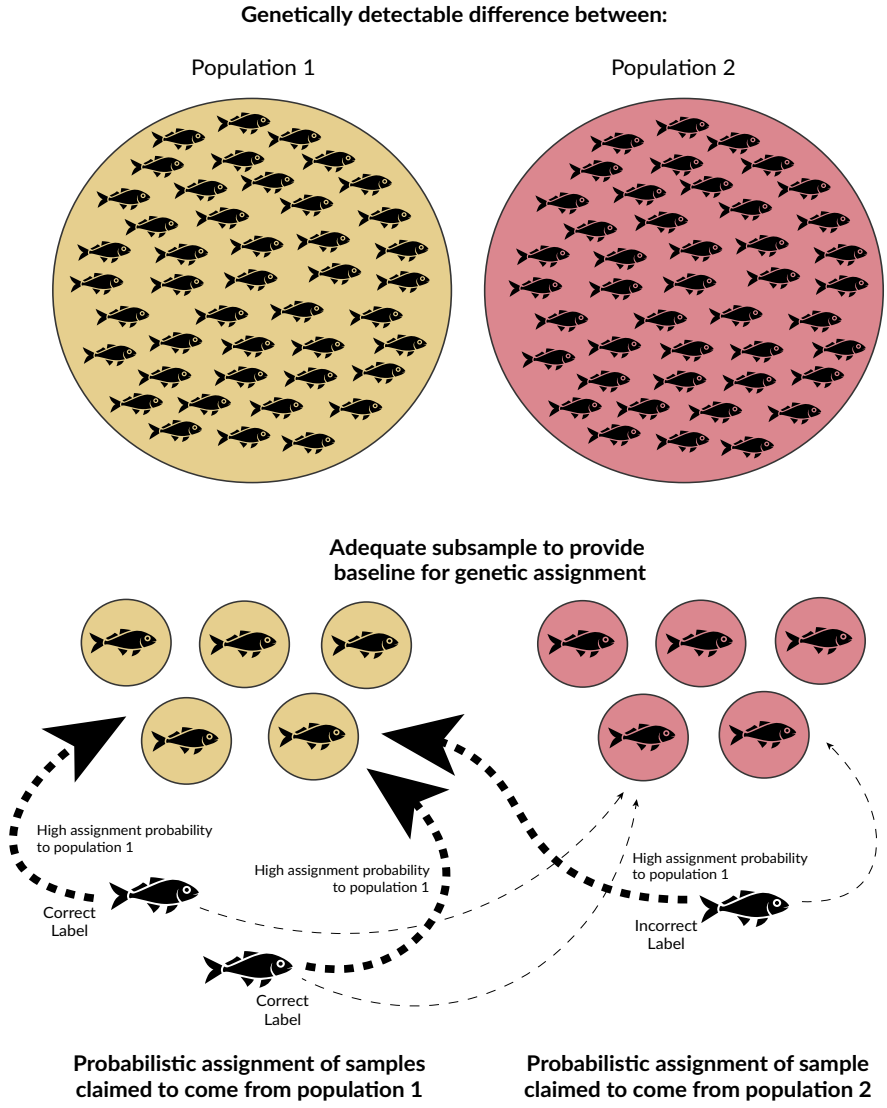
Over the last two decades, genetic methods have helped uncover substitution of species in the seafood industry worldwide and have exposed species mislabelling as a prevalent phenomenon that greatly hampers traceability efforts (Kroetz *et al.*, 2020; Miller and Mariani, 2010; Wong and Hanner, 2008; Jacquet and Pauly, 2008). This worrying trend has prompted exploration on the rate of species mislabelling across the seafood supply chain, illustrating that some species (Cawthorn *et al.*, 2018) or some sectors, such as the restaurant industry (Christiansen *et al.*, 2018; Vandamme *et al.*, 2016), may be at higher risk of product substitution than others. Media coverage, consumer awareness and the involvement of non-governmental organizations have led the industry and public authorities to tighten their control and have contributed to increased traceability standards and decreased species mislabelling trends in the seafood industry (Mariani *et al.*, 2015).

Compared to species mislabelling, the verification of the geographical catch location of products is much less prevalent in forensic testing of seafood (Ogden, 2008). This type of testing is more labour intensive and expertise dependent, and relies on probabilistic principles, which often make it less suited for denouncing instances of mislabelling, particularly if fraud is suspected (Nielsen, 2016). Yet given the disparate levels of conservation status of fish stocks within species, which often require marked differences in quota allocations, verifying potential instances of fraud on a spatial scale is highly relevant. Indeed, some species may be composed of both well-managed sustainable stocks and depleted stocks, and the sustainable management of these stocks depends greatly on compliance with allocated quotas. If these quotas are not respected or fish are harvested in adjacent poorly managed stocks, substitution of catch location may be one way of letting these illegally caught specimens enter the supply chain (Nielsen *et al.*, 2012a; Nielsen *et al.*, 2012b). Despite the ecological importance of verifying catch locations, few studies have investigated the issue, leaving the door open to malpractice (Martinsohn *et al.*, 2019).

USING GENETIC TOOLS FOR PROVENANCE TESTING

Testing for mislabelling of the geographical catch location implies that a given claimed location is associated with a specific fish stock for which biological boundaries should match geographical stock-management boundaries (Reiss *et al.*, 2009). When it comes to genetic methods, these boundaries very much depend on the level of reproductive isolation between stocks, which will afford each stock a slightly different genetic make-up (Ogden, 2008; Ogden and Linacre, 2015). Part of the reason that genetic tools are not commonly used to determine catch location is that the populations of interest are not always fully reproductively isolated and may not always display detectable, unambiguous, diagnostic genetic characters. In some circumstances, reproductively isolated populations exist, but they do not match the stock assessment and management boundaries (Reiss *et al.*, 2009). For this reason, identifying catch location requires more upstream evaluation on the efficacy of genetic tools, and results will always come with some level of probabilistic uncertainty (Nielsen, 2016). In simplest terms, DNA-based identification of geographical origin is founded on two prerequisites: i) the existence of detectable, consistent genetic differences between populations inhabiting fished areas, and ii) the availability of "baseline" specimens whose genotypes are representative of those populations. Figure 2 provides a generalized view of this process.

FIGURE 2
Schematic representation of the probabilistic assignment to reference populations

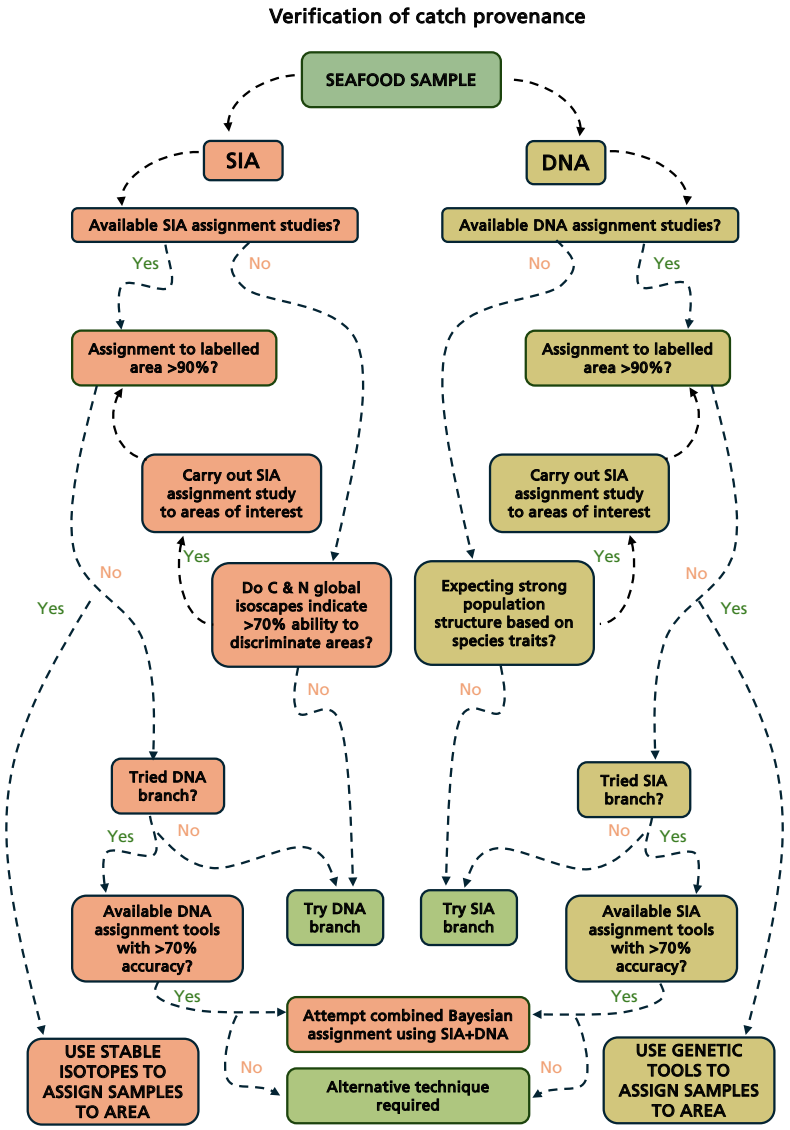


Note: The thick arrows represent the most likely population assignments and the thin arrows represent the least likely population assignments.

USING STABLE ISOTOPE TOOLS FOR PROVENANCE TESTING

While DNA-based tools depend on evolutionary principles, stable isotope analyses are reliant on spatial variations in chemical tracers. The organic isotopic composition varies across space and is therefore reflective of the geographical locations an organism has inhabited and where it has foraged (Bowen, 2010). These tracers cannot identify the population of origin of an organism, but they can give insight into the general region. In order to be informative and evaluate catch location, results must be compared against isoscape models and mapping (Cusa *et al.*, 2022). Both genetic tools and stable isotope analysis have benefits and disadvantages in the evaluation of catch location and in identifying instances of geographical mislabelling. Figure 3 offers a pathway for decision-making when attempting to trace an individual back to its population of origin.

FIGURE 3
Decision tree to evaluate whether genetic tools or stable isotope analysis might be more adapted to identify the geographical catch location of a specimen



Note: SIA: stable isotope analysis, DNA: deoxyribonucleic acid, C&N: carbon and nitrogen.
Source: Adapted from Cusa, M., St John Glew, K., Trueman, C., Mariani, S., Buckley, L., Neat, F. & Longo, C. 2022. A future for seafood point-of-origin testing using DNA and stable isotope signatures. Reviews in Fish Biology and Fisheries, 32(2): 597–621. https://doi.org/10.1007/s11160-021-09680-w

THE CASE IN THE LITERATURE

In 2014, R. Ogden and G. Murray-Dickson published a report for the Department for Environment, Food & Rural Affairs of the United Kingdom for which they developed genetic markers to evaluate the geographical population of origin of four species of fish: Atlantic cod, European hake (*Merluccius merluccius*), common sole and Atlantic herring (*Clupea harengus*). Based on thousands of voucher specimens, they identified highly diagnostic SNPs with the goal of reducing both the cost and complexity of the

method to be employed while maintaining high levels of confidence for enforcement purposes (Ogden and Murray-Dickson, 2014).

A panel of 1 290 SNPs for 942 Atlantic cod was reduced to a mere 9 SNPs to assign cod back to either the Northeast Arctic or the North Sea with 98 percent assignment accuracy. Despite the method's efficacy and potential for evaluating point-of-origin fraud (Nielsen *et al*., 2012a), it took another decade for it to be used in the context of mislabelling verification in Europe (Cusa *et al*., 2025).

In 2020, the first study was conducted to investigate the level of mislabelling of marketed Atlantic cod provenance using the SNPs identified by Ogden and Murray-Dickinson (2014). Atlantic cod is well suited for this type of analysis as, despite being a migratory fish, it has well-defined reproductive and population boundaries (Barth *et al*., 2017; Bekkevold *et al*., 2015; Hemmer-Hansen *et al*., 2013; Nielsen *et al*., 2012a) and those boundaries match to a great extent the subzones of the International Council for the Exploration of the Sea in the Northeast Atlantic (FAO 27) (FAO, 2025b). Atlantic cod also happens to be a fish of huge economic value with a history of overexploitation, mismanagement and stock collapse. Evaluating the reliance of the traceability system for cod is therefore quite relevant as the species is prone to overfishing, and various stocks will require drastically different management plans, as shown by at least ten different cod stocks currently assessed by the International Council for the Exploration of the Sea (ICES, 2025a).

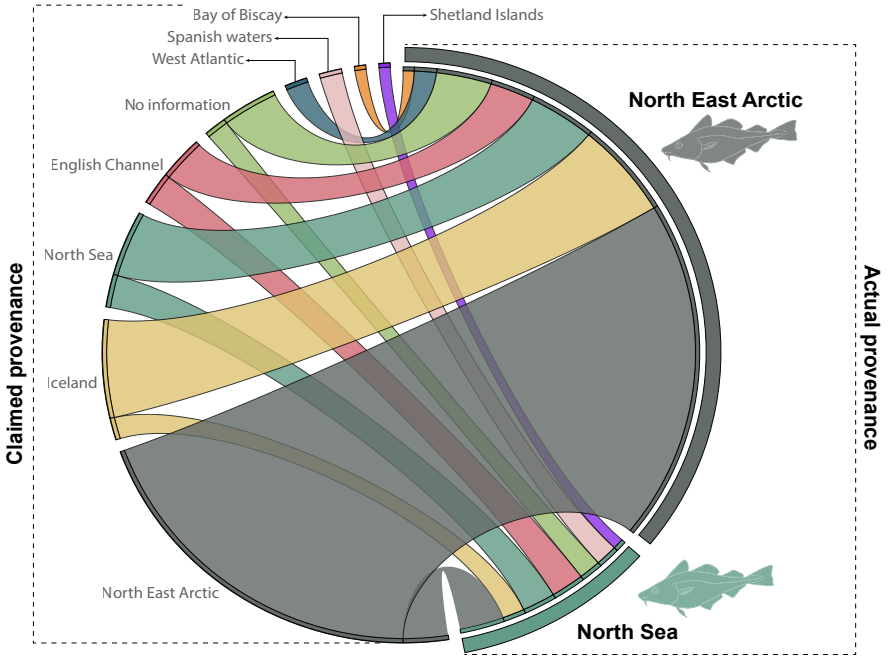
Today, most of the Atlantic cod consumed is caught in the Northeast Arctic, particularly in the Barents Sea, the Norwegian Sea, and Iceland and Faroes grounds. North Sea Atlantic cod stock is also targeted particularly by local coastal states such as Denmark, Norway and the United Kingdom (ICES, 2022), and since the case study was conducted, the latest assessment prompted a "zero catch" advice for 2026 (ICES, 2025b). Given the current context of recent cod stock fluctuations in the Northeast Atlantic (which led to various stock-specific seasonal bans and increasingly stringent regulations imposed by the European Union) testing for geographical-provenance mislabelling of cod is timely.

The case study described here, which is further described in Cusa (2022) and Cusa *et al.* (2025), focused on developing a parallel sequencing protocol to segregate between cod populations from the Northeast Arctic (Barents Sea, Norwegian Sea, Bear Island and Spitzbergen) and cod populations from the North Sea using the nine diagnostic SNPs identified by Ogden and Murray-Dickson (2014). Following the development of this technique, the study tested it on cod samples of known origin to evaluate the efficacy and accuracy of the method in assigning specimens back to their population of origin. This was followed by the first pan-European cod-provenance market study using these genetic tools on cod samples from both fishmongers and supermarkets, sourced in France, Germany, Spain and the United Kingdom.

Only 2 percent of the market samples were not identified as Atlantic cod, but around 30 percent of them were mislabelled in terms of geographical origin, with two out of three (67 percent) specimens sold with a North Sea label actually coming from the Northeast Arctic. This study illustrates the urgent need to further examine the rate of geographical-provenance mislabelling across species and regions. It has been argued that species mislabelling may gradually decrease due to public awareness and improvement in traceability standards. This might be true for species identification and labelling, but geographical origin is currently grossly underexplored, certainly removed from the average consumer's attention, and most likely exposed to fraudulent activities.

Pushing this examination further illustrates that as much as 50 percent of the total samples may have been mislabelled, though this remains to be confirmed with a panel of SNPs that could more specifically discriminate between more than two cod populations (Figure 4).

FIGURE 4
Circos plot illustrating claimed provenance of cod products from retailers and actual provenance



Note: Claimed provenance provided by retailers either verbally or on packaging. Actual provenance evaluated using GENECLASS2.0 with over 80 percent assignment accuracy and minimum amplification of eight SNPs.

SCALE AND GLOBAL INCIDENCE OF THE CASE

Little research has been conducted regarding the issue of provenance mislabelling of marine species. As such, its scale is unknown. This illustrates further the need to investigate these methods of determining geographical provenance. As for Atlantic cod, the mislabelling of point-of-origin was widespread across several European Union countries, though some countries, such as France and Spain, performed worse than others that displayed higher levels of accurate labels, such as the United Kingdom and Germany (Cusa, 2022).

PUBLIC-HEALTH ASPECTS AND OTHER IMPLICATIONS

Mislabelling of seafood geographical origin can have important public-health implications. Consumers rely on accurate labelling to make environmental and ethical choices, and to avoid health risks that might be associated with fish from certain regions. Seafood from regions that have high pollution or contaminant loads may cause health concerns related to the presence of, for example, heavy metals, polychlorinated biphenyls, parasites, or pathogens. By consuming mislabelled specimens, vulnerable people such as pregnant women and children may unknowingly consume fish from regions not recommended for them. Failures in the traceability of geographical catch location undermines consumer protection and highlights the need for robust implementation and enforcement of verification mechanisms throughout the seafood supply chain.

TOOLS TO PREVENT THE ISSUE

While DNA-based species identification tools are well established and begin to be applied with some regularity, population-level geographical assignment methods are lagging, except for a handful of valuable and emblematic species. Greater impetus in characterizing the spatial genetic constitution of a much wider set of commercially important species is needed, to develop panels of SNP markers able to trace seafood products to the most likely region of origin. With current technologies – which require, at a minimum, the sampling of individual fish from the most fished areas, the existence of a high-quality reference genome, and the selection and validation of geographically diagnostic markers – the process of validating an operational toolkit for just one species may take as long as 2 years. Significant investments are therefore required to make these tools a reality for the hundreds of species currently fuelling the global seafood market.

In the long run, it should be convenient to invest in novel technologies that may allow massive-throughput screening of environmental DNA (eDNA) samples, which contain fragments informative of the genetic make-up of several species from a certain area, thereby allowing quicker reconstruction of the genetic reference maps for key species and stocks across regions. Such progress will only be possible alongside significant development of nimble computational solutions (one area where artificial intelligence could be a force for good) to process such large datasets and link them to market samples.

Irrespective of how powerful, available and user-friendly these genetic tools will be in the near future, they will primarily be mechanisms of control and verification. To guarantee a sustainable and transparent supply chain that accurately tracks geographical provenance, greater progress must be made towards improved stakeholder dialogue, legislation and ethical responsibility, so that all sectors can function with greater coordination and a renewed awareness for the needs of consumers, fishers and the natural environment.

CONCLUSIONS AND RECOMMENDATIONS

Concerns over the environmental, economic and human-health impact of seafood mislabelling have provided momentum for a large body of studies to explore seafood markets worldwide for potential instances of misidentification and fraud. Thanks to groundbreaking initiatives, such as the International Barcode of Life Consortium, and improved genetic technologies, these types of studies have flourished over the last decade. Yet, whereas identifying species using DNA has become a routine examination, tracing a marketed specimen back to its population of origin is falling behind, particularly for mobile organisms like fish. The technique developed to determine the geographical origin of Atlantic cod described in this case study, reveals that not only is it possible to evaluate catch location in a context of enforcement and monitoring, but it should become a regular exercise. The case study reveals that mislabelling catch location is much more prevalent than mislabelling species. These results illustrate a failure in traceability along the supply chain, with reasons that are likely diverse and complex. If this is true for a well-studied, widely distributed, iconic species such as cod, it is reasonable to expect that the situation may be even worse for hundreds of other less prominent species. Irrespective of the causes of such a high mislabelling rate, it seems apparent that, despite incentives for improved seafood transparency, the European Union Commission and other large translational organizations must strengthen legislation and step up enforcement through verification points along the supply chain. Given the poor state of some stocks, authorities and retailers should be able to verify seafood provenance, and customers should be given the ability to choose where their seafood comes from using reliable labels.

Case study 11. Academic and government initiatives for DNA-based identification of fish mislabelling in the neotropics: case studies in Brazil

The Barcode of Life Database (BOLD) is a public, cloud-based data platform developed at the Centre for Biodiversity Genomics in Canada that allows for searching over 9.7 million public records using multiple search criteria, including geography and taxonomy. BOLD contains records of more than 300 000 fish COI DNA sequences, encompassing 293 938 records of the class Actinopterygii, 22 177 of Elasmobranchii and 202 reference sequences of Sarcopterygii. Thus, with the development of a standardized database of fish COI sequences collected throughout the world, it is now possible to use this data to molecularly identify most species and pinpoint mislabelling and fraudulent commercialization of fish, including products from regions with highly biodiverse ichthyofauna, such as the neotropics (Barbosa *et al*., 2021; Calegari *et al*., 2019; Carvalho *et al*., 2017; Souza *et al*., 2021). For example, in 2005, using DNA barcodes (that is, analysing about 650 base pairs of the COI mitochondrial gene) and the BOLD database, a state regulatory agency conducted an investigation in the markets of Florianopolis, in southern Brazil, aimed at detecting mislabelled seafood products (including fresh filets as well as seafood in cooked meals), which, if found, resulted in financial penalties for the retailers (Carvalho *et al.,* 2005). In this investigation, cases of mislabelling were found in 24 percent of the samples, with expensive species (such as flounder, pink cusk-eel and cod) being substituted by cheaper species (such as basa and Alaska pollock). However, the implementation of such regulatory programmes using DNA-based identification methods has not discouraged deliberate substitution in this market. For instance, in 2017, it was reported that 30 percent of samples from fisheries and 26 percent of samples from Japanese restaurants, in the same city, were mislabelled (Staffen *et al*., 2017).

A broader government initiative to use DNA barcoding as a standardized method for routine and systematic regulation of seafood products was implemented by the Brazilian Ministry of Agriculture, Livestock and Food Supply – the ministry responsible for ensuring accurate labelling of foodstuff at the federal level. They analysed fish products from 14 Brazilian states as well as imports from 8 countries, and reported a mislabelling rate of 17.3 percent (Carvalho *et al*., 2017a).

Further, with the development of new technologies, such as high-throughput DNA sequencing, it is possible to identify species mixtures using a powerful approach called DNA metabarcoding or food metagenomics (Carvalho *et al.,* 2017b). Fish-species mixtures are common within processed cod products, such as fish cakes, and are popular around the world, as well as being expensive in Brazil. Cod products are very prone to mislabelling since, under Brazilian legislation, only four species can be legally labeled "cod" (or *bacalhau,* in Portuguese): Pacific cod (*Gadus macrocephalus)*, Atlantic cod (*Gadus morhua)*, Greenland cod (*Gadus ogac*) and polar cod (*Boreogadus saida*). Using DNA metabarcoding, Carvalho *et al.* (2017) identified species that were used to produce processed fish products (such as cod pieces, frozen cakes, vacuum-packaged cooked meals, a restaurant dish and fast-food cod cakes) and sold (and labelled) as "cod". A mixture of two or more species, including non-cod fish were found in 31 percent of all products.

Case study 12. Novel method for authenticating the geographical origin of tiger prawn

INTRODUCTION

Seafood is a crucial tradeable commodity and serves as a major source of animal protein for many people worldwide (Asche *et al.*, 2015). The global seafood industry generated an estimated USD 151 billion in 2020 (FAO, 2020). Seafood also plays a vital role in global food security, with consumption increasing by 3.5 percent per annum over a 57-year period to 2022, outpacing the population growth rate of 1.8 percent (Issifu *et al.*, 2022; FAO, 2018b). With the global population projected to reach approximately 10 billion by 2050 (United Nations, 2015), the demand for all types of food, including seafood, will rise significantly. To meet this demand, the seafood industry must increase production.

Currently, aquaculture is responsible for the majority of seafood production, surpassing capture fisheries and wild harvests (FAO, 2018b). This increase in production is likely to boost the value of the global seafood industry through heightened imports and exports. However, the increased demand and higher profitability of seafood can motivate dishonest market-chain actors to intentionally mislead consumers for greater profit. Food fraud, the deliberate misrepresentation or adulteration of food products for financial gain, is a growing global concern with significant economic, health and social impacts (Bannor *et al.*, 2023; Spink and Moyer, 2011). Globally, food fraud affects various products, including food, meat, dairy, and seafood, and costs the global economy billions of dollars annually (European Commission, 2018).

Food fraud in the seafood industry can take many forms, including mislabelling species or origin, adding non-declared substances, and substituting high-value species with lower-value species, among other types of fraud (Lawrence *et al.*, 2022). This not only deceives consumers but also poses health risks, as some substituted species may contain allergens or toxins. Falsified provenance information can impact consumers' and honest producers' interests and fair trade, and it can have food-safety implications, for instance, in the case of fishing in polluted environments. Additionally, food fraud undermines consumer trust and can damage the reputation of legitimate businesses.

THE CASE IN THE LITERATURE

Seafood is the fourth most consumed protein in Australia, after red meat and poultry and processed meat (Sui *et al.*, 2017). Australian seafood production has a projected value of AUD 3.6 billion (approximately USD 2.3 billion) for 2023-2024 (DAFF, 2024a) and is renowned locally and internationally for its premium quality. However, this prestige makes the industry vulnerable to adulteration, mislabelling, substitution and other forms of market-chain manipulation. To safeguard the import and export supply chain against fraudulent activities, accurate and reliable methods for determining seafood provenance are essential.

In Australia, a study revealed that 11.8 percent of seafood products were mislabelled, with sharks, rays and snappers having the highest mislabelling rates (Cundy *et al.*, 2023). Poor labelling practices, including the use of vague common names, contribute to this issue (Cundy *et al.*, 2023). Factors facilitating fraud include inadequate testing regimes, unclear definitions and regulatory gaps. Weak labelling regulations and ambiguous naming conventions also contribute to high mislabelling rates. For example, in Australia, only 25.5 percent of products are labelled at the species level, hindering consumer choice for sustainable options (Lindley, 2021).

Australia's National Agricultural Traceability Strategy 2023 to 2033 emphasizes the importance of strong traceability credentials to meet emerging requirements, support product integrity claims, and remain competitive in the complex international trading environment (DAFF, 2024b). Recently, the government included mandatory

country-of-origin labelling (CoOL) (Australian Government, 2016) for seafood in hospitality settings. This initiative will help consumers make educated and informed purchasing decisions for seafood in hospitality settings, aligning with their personal preferences.

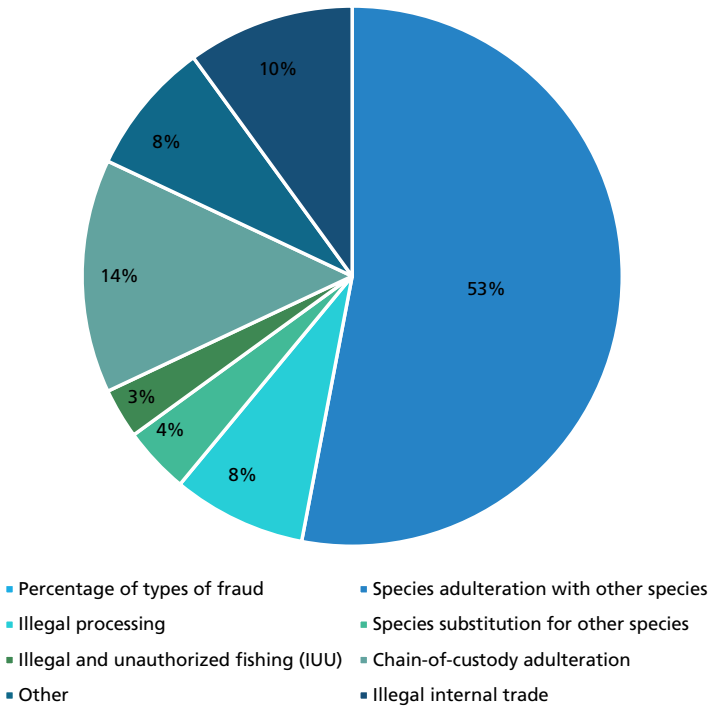
To address traceability requirements in the seafood supply chain, the Australian Nuclear Science and Technology Organisation, in collaboration with the University of South Wales and the Sydney Fish Market, has developed two innovative solutions. The first approach involves laboratory-based techniques using isotopic and multi-elemental analyses of seafood. The second approach utilizes X-ray scanning technology through a portable, handheld device to provide real-time sample analysis in the marketplace, offering provenance solutions as a deterrent against fraud.

Scale and global incidence of the case

Globally, seafood fraud falls into three main categories: origin, adulteration and ethical trade (Fox *et al.*, 2018). Lawrence *et al.* (2022) further elaborates on these categories, showing that fraud cases in the United States and the European Union include species adulteration by adding other, undisclosed and often cheaper products (53 percent), illegal international trade (10 percent), illegal and unauthorized fishing (3 percent), and species substitution (4 percent), which is related to chain-of-custody fraud (Figure 5).

FIGURE 5

Select types of food fraud and percentage of occurrence in the United States and the European Union



Source: Adapted from Lawrence, S., Elliott, C., Huisman, W., Dean, M. and van Ruth, S. 2022. The 11 sins of seafood: Assessing a decade of food fraud reports in the global supply chain. Comprehensive Reviews in food Science and food Safety, 21(4): 3746-3769. https://doi.org/10.1111/1541-4337.12998

A crime script analysis study by Lawrence *et al.* (2024) examined ten court cases involving various seafood-related fraud cases in the United States and the United Kingdom. Six cases involved mislabelling imported goods as local, with one also involving wild and aquaculture substitution. Three cases involved species substitution, two involved miscellaneous label swaps, one involved illegal import, and one involved illegal fishing. The study highlights the circumstances and methods used by perpetrators to commit fraud, emphasizing the risks posed by incomplete traceability systems, inadequate certification standards, lack of provenance-identification technologies within the industry and enforcement agencies, and insufficient consumer knowledge in fraud risk-assessment strategies.

A study by Warner *et al.* (2013) found that, on average, one in three seafood products tested in the United States were mislabelled. Despite this high rate, only 1 percent of all seafood entering the United States is reportedly tested by authorities for mislabelling (Fox *et al.*, 2018). Similar cases have been reported globally, from Europe to Asia, where seafood substitution and mislabelling are common occurrences (Fox *et al.*, 2018; Buck, 2007).

A study by Sumaila *et al.* (2020) explored IUU fishing, revealing that foreign vessels "launder" seafood by substituting lower-value species for higher-value species or altering the origin of the catch. The illegal trade of fish caught off the coasts of Africa, Asia and South America costs between USD 26 billion and USD 50 billion, with tax losses to the rightful nations amounting to between USD 2 billion and USD 4 billion.

In 2014, in a case of fraudulent misrepresentation of origin, imported, farmed shrimp from Ecuador, Mexico and elsewhere was being sold as a wild-caught, local product of the United States (Lawrence *et al.*, 2024). The case *United States of America v. Alphin Brothers Inc.*, 2014, was prosecuted through the US Federal Court and involved 13 450 lbs of shrimp, resulting in USD 100 000 in fines for the company, which also faced a 3-year probation.

In December 2024, Europol and law enforcement agencies from France, Portugal and Spain dismantled a seafood-fraud ring operating across multiple jurisdictions. The ring was illegally harvesting contaminated molluscs linked to hepatitis and selling them in local markets. Authorities seized 30 tonnes of produce, valued at EUR 10 million (approximately USD 10.8 million). The same group was also involved in poaching and smuggling glass eels, and the case was connected to human trafficking (European Union Agency for Law Enforcement Cooperation, 2024).

Public-health aspects and other implications

Seafood fraud is a growing international problem that significantly impacts food safety. Successful seafood businesses, including those involved in imports and exports, require stringent measures to ensure food safety, quality and product authentication. Factors such as geographical locations, production methods (both aquaculture and wild catch), processing, accurate labelling and handling all influence the safety matrix of seafood products and can pose health risks to consumers. Mislabelling and species substitution not only undermine consumer trust but also have economic, environmental (Cundy *et al.*, 2023), and health consequences.

The presence of contaminants, pesticides and antibiotics (Alberghini *et al.*, 2022; Bondad-Reantaso *et al.*, 2023) can lead to various health issues for consumers, including antibiotic resistance and exposure to harmful chemicals. The overuse and misuse of antibiotics in aquaculture production have resulted in the emergence of resistant microorganisms and antimicrobial residues, posing a public-health challenge (Bondad-Reantaso *et al.*, 2023; Reverter *et al.*, 2020). Several reports have indicated that seafood commodities have been subjected to import refusals due to food-safety concerns associated with drug residues (Gale and Buzby, 2009).

Contaminants such as heavy metals (Ray and Vashishth, 2024), perfluoroalkyl and polyfluoroalkyl substances (Christensen *et al.*, 2017), and biogenic amines (BAs) in

seafood pose significant health risks when consumed (Chaidoutis *et al*., 2019). For example, certain species of fish (such as those in the Scombridae family) are more prone to harbouring histamine-producing bacteria, if not handled or stored appropriately (Lunestad *et al*., 2011). Other species such as mahi-mahi, anchovy, amberjack, marlin, bluefish, herring and sardine have been implicated in cases of scombroid poisoning after consumption; and fish with dark flesh containing free histidine can contain elevated levels of histamines (Taylor *et al*., 2025).

Microplastic and nanoplastic contamination of fish and shellfish poses risks to food webs and humans, as some degraded plastics found in edible and non-edible tissues of sea organisms (Akoueson *et al*., 2020) contain harmful chemicals. The level of toxicity to biota and humans (via consumption) depends on the physical and chemical properties of the polymer (Casagrande *et al*., 2024). Microplastic and macroplastic particles bioaccumulate through the food chain (Danopoulos *et al*., 2020), with some chemicals linked to diseases in humans affecting the hypothalamus, thyroid, testes and ovaries. (Plastic particles have been found in human urine, faeces, placenta and breast milk.) Moreover, plastic particles absorb environmental contaminants, which can persist through the food chain (Taylor *et al*., 2025).

Allergens in seafood are another significant health risk. A study by Dorney *et al*. (2024) found that the geographical location of capture or aquaculture influenced the allergenic protein profiles of black tiger shrimp (*Penaeus monodon*), one of the most farmed and consumed shrimp species worldwide. Accurate labelling and declaration of potential allergens in seafood products are emerging public-health concerns. Ciguatera poisoning, caused by harmful algal blooms attributed to environmental factors and climate change, is another food-safety concern. It is increasingly reported in large reef fish and is the most common foodborne illness related to finfish consumption globally (Friedman *et al*., 2008; Kumar-Roiné *et al*., 2011).

Biosecurity breaches can have devastating economic consequences for the seafood industry, potentially wiping out entire sectors. For example, Australia prohibited the import of uncooked prawns from Asia in early 2017 following an outbreak of the deadly white spot disease (Do and Vanzetti, 2018). Such incidents highlight the importance of stringent biosecurity measures to protect the industry and ensure the safety of seafood products.

TOOLS TO PREVENT THE ISSUE

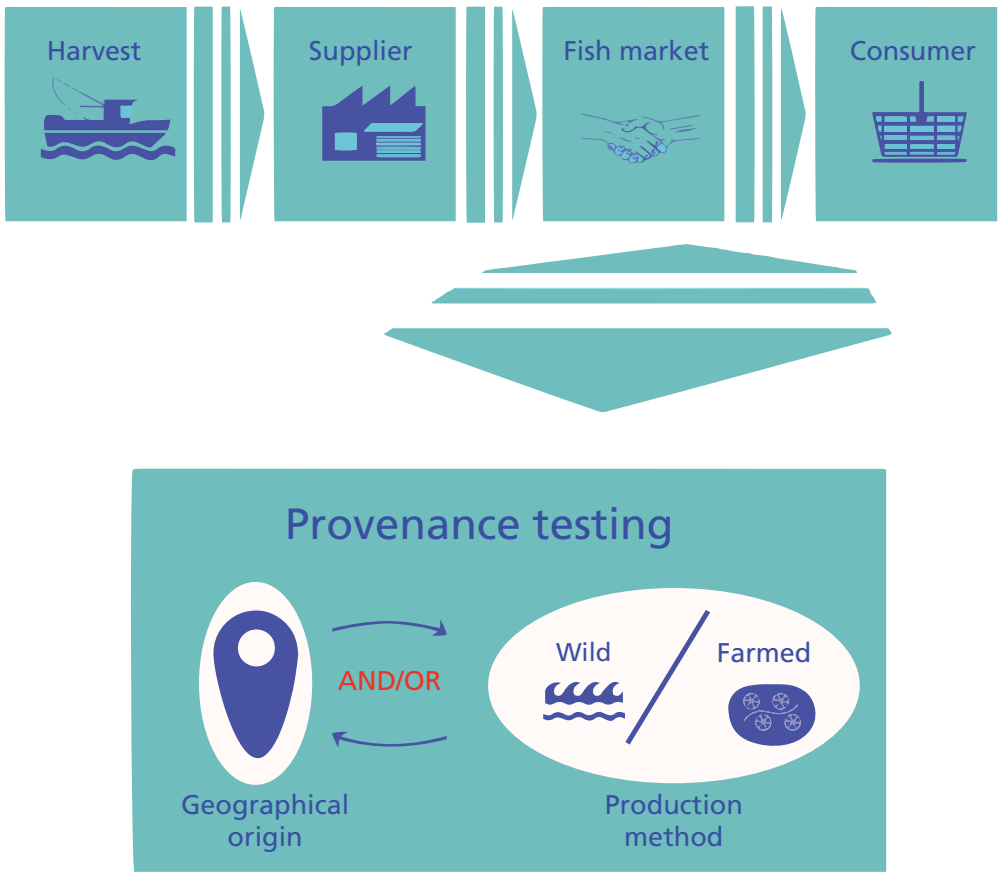
Current methods to determine seafood provenance include stable isotope ratio analysis, gas chromatography and liquid scintillation counting. Multi-element analysis techniques such as inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Anderson *et al*., 2010), LA-ICP-MS (Sorte *et al*., 2013), ICP-MS, inductively coupled plasma optical emission spectroscopy (ICP-OES), XRF, ion-beam analysis and neutron-activation analysis, are also used (Gopi *et al*., 2019a, 2023). Other techniques involve NMR spectroscopy (Longobardi *et al*., 2015, Lolli and Caligiani, 2024) and high-resolution mass spectrometry for metabolomics and proteomics. Rapid screening methods include ion-mobility spectrometry (Arce *et al*., 2014), near-infrared spectroscopy (Woodcock *et al*., 2008; Zao *et al*., 2024), hyperspectral imaging, Raman spectroscopy (Damiani *et al*, 2020), DNA and fatty-acid profiling (Ricardo *et al*., 2015), and blockchain methods (Yiannas, 2018). Each of these analytical techniques has its own advantages and disadvantages (Gopi *et al*., 2019a; Hassoun *et al*., 2020b) making it challenging to recommend a single method for detecting seafood fraud.

A multilayered approach has been tested and proven successful for authenticating the origin of seafood. This method combines stable carbon and nitrogen isotope data from continuous-flow isotope ratio mass spectrometry with multi-elemental data from Itrax X-ray fluorescence scanning and ion-beam analysis. The data generated by these analytical tools are then analysed using artificial-intelligence-based machine-learning

models to authenticate the geographical and production origins of seafood. This combined methodology shows promise for seafood provenance, demonstrating advantages in identifying the geographical source and production methods (such as farmed or wild-caught) of seafood with over 80 percent accuracy (Gopi *et al.*, 2018, 2019b, 2019c, 2022). By demystifying supply chains, this approach helps protect consumers against fraud, providing greater transparency and reliability in the seafood industry. The integration of these techniques not only enhances the accuracy of provenance identification but also supports the enforcement of certification standards and traceability systems.

The following two case studies illustrate the use of lab-based isotopic and elemental analysis, along with portable handheld XRF scanning techniques, to authenticate the origins within the supply chain (Figure 6).

FIGURE 6
Block diagram of the seafood supply chain and the authentication of origin using provenance technology

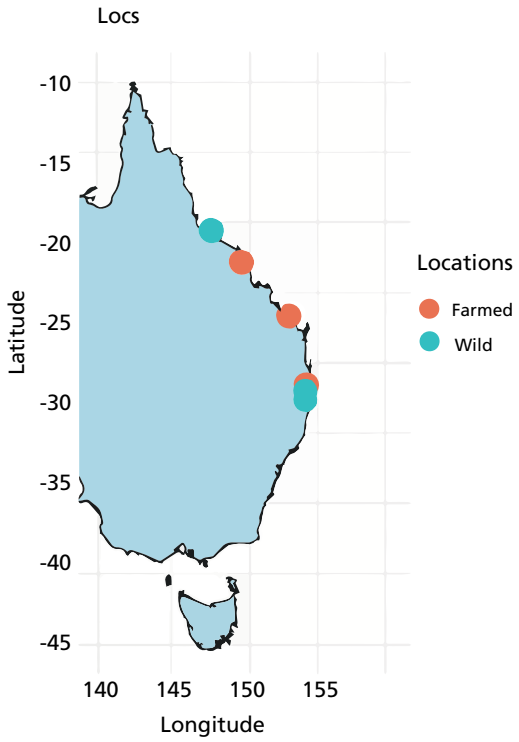


LABORATORY-BASED ANALYTICAL METHODS FOR PROVENANCE DETERMINATION

This method relies on the combined use of isotopic and multi-elemental analyses, along with machine learning for data analysis, to authenticate the origin of seafood. This comprehensive approach ensures accurate identification of the geographical and production origins of seafood.

To test the capacity of isotopic and elemental analysis for provenance, nine (n = 9) tiger prawn (*P. monodon*) samples from each of eight locations (four farmed and four wild caught), totalling 72 samples, were collected across the eastern seaboard of Australia (Figure 7). The samples were transported frozen to Australian Nuclear Science and Technology Organisation. Once thawed, a 2 cm² cube of dorsal muscle tissue was removed from each sample, cleaned and dried at 60 °C. The dried samples were then ground into a fine powder using a titanium-ball mill grinder for isotopic and elemental analyses (Gopi, 2022).

FIGURE 7
Sample collection locations



Note: Red circles indicate the locations of farmed samples. Blue circles indicate the locations of wild samples. Overlapping circles represent proximity between locations.

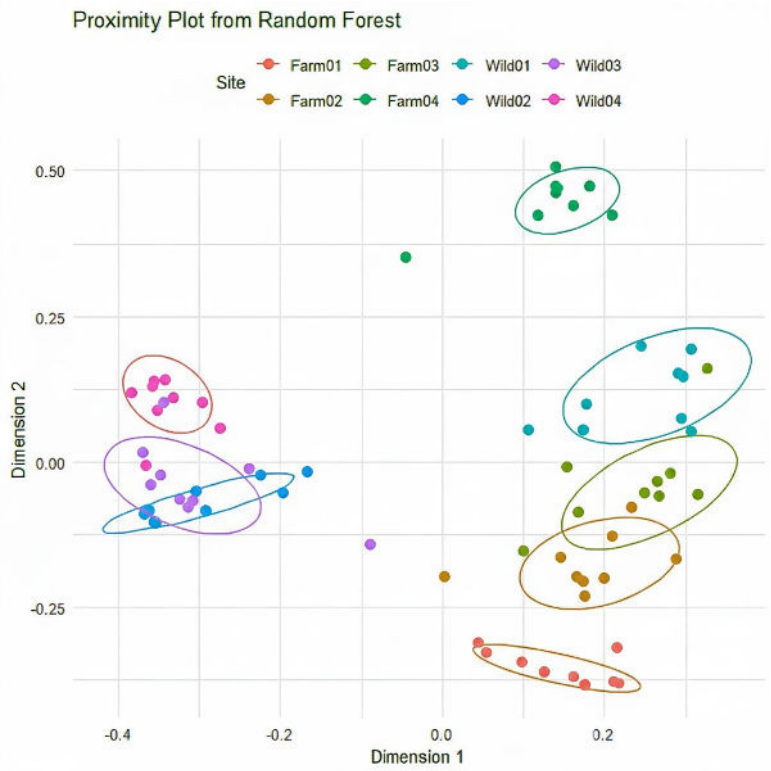
Stable carbon- and nitrogen-isotope analyses were conducted using a Thermo Scientific Delta V Plus continuous-flow isotope-ratio mass spectrometer, interfaced with a Thermo Fisher Flash 2000 HT EA elemental analyser. Additionally, the elemental composition was determined using an Itrax Micro XRF core scanner with a molybdenum tube (Gadd *et al.*, 2018), which produced the relative abundance of 31 different elements (Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Y, Zr, Cd, Sn, Sb, Nd, Hf, Pb, Bi, At and U) present in the samples.

A machine-learning model, specifically Random Forest, was applied to the stable isotope and multi-elemental composition data to determine the provenance of tiger

prawns. In machine learning, a model is trained on labelled authentic data to learn how to classify new, unseen data into the appropriate group. The Random Forest algorithm uses an ensemble of decision trees to improve the predictive accuracy of test samples (Liaw and Wiener, 2002).

The results of the lab-based combined stable-carbon and nitrogen isotope and multi-elemental analysis, along with the application of machine learning, effectively distinguished prawns from different origins. There is some overlap between wild-caught prawns from regions 2 and 3 (Figure 8). The overall prediction accuracy for classifying geographical origins, including farmed and wild production methods, was 86 percent using the Random Forest model.

FIGURE 8
Proximity plots from Random Forest showing the degree of separation of samples from different locations



PORTABLE SCANNING METHOD FOR PROVENANCE

Laboratory-based sample analysis is often time consuming and requires specialized instruments. To address this, portable, handheld XRF scanning techniques have been developed as a first line of defence for seafood provenance, ensuring supply-chain integrity and benefiting the seafood industry and regulatory authorities (Malo *et al.*, 2023; Martino *et al.*, 2023). Portable, handheld, X-ray fluorescence (XRF) scanning is a fast, non-destructive and user-friendly technique for elemental analysis (Bosco, 2013; Shackley, 2018; Gałuszka *et al.*, 2015). This method scans fresh seafood on the market floor to identify chemical markers of specific environments, regions and farming processes to ensure high labelling accuracy, helping consumers make informed purchasing decisions (Martino *et al*, 2023).

To authenticate the origin of seafood, market-size tiger prawns were collected from 13 harvest sites across three geographically distinct states in Australia: New South Wales, Queensland and Northern Territory, representing both farmed and wild-caught origins. New South Wales is known for its temperate climate, Queensland for its subtropical conditions, and Northern Territory for its tropical environment, providing a diverse range of samples. The samples were transported frozen to the Australian Nuclear Science and Technology Organisation laboratory and, once thawed, analysed through a handheld XRF scanner. The edible muscle tissue of each tiger-prawn sample was scanned in its raw state.

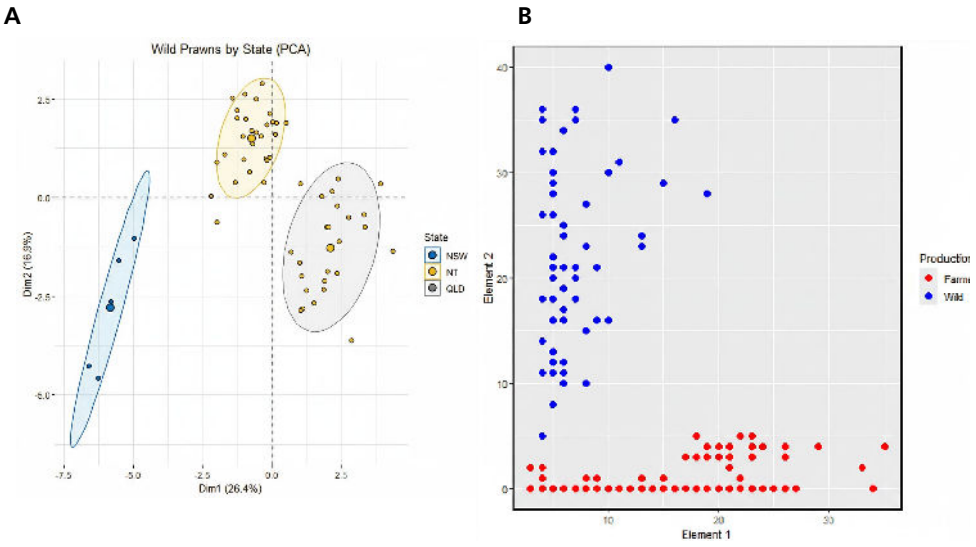
The portable, handheld XRF (Model VANTA™) features a sensitive large-area silicon drift detector, a 50 kV X-ray tube, and a rhodium (Rh) anode. The scanner can obtain elemental concentration from Mg to U and light elements through scanning of samples.

The instrument was placed within a shielded workbench stand, a portable enclosure that ensures no external radiation is present. However, the instrument can also be used in a handheld manner, scanning the sample directly. Measurements were obtained using the "3-beam" setting (40kV, 10kV and 50kV respectively), with a 60-second exposure time per beam, totalling a 180-second scan duration per sample (Malo *et al.*, 2023). The resulting elemental profiles were then analysed using unsupervised statistical classification and an artificial-intelligence-driven supervised machine-learning model (Liaw and Wiener, 2002).

Unsupervised statistical analysis (principal component analysis, or PCA) revealed that tiger prawns harvested from different states were grouped separately (Figure 9[A]) and exhibited significantly different elemental profiles. Similarly, analysis indicated that tiger prawns originating from the wild ocean were distinct and separated from their farmed (river) counterparts (Figure 9[B]).

FIGURE 9

(A) PCA results showing a separation between samples from New South Wales, Northern Territory and Queensland; (B) Scatter plot demonstrating a good distinction between farmed (river) and wild (ocean) samples for select elements



When analysing these elemental data through machine-learning algorithms, the results showed an accuracy of 88 percent for determining geographical origins and 96 percent for distinguishing between farmed and wild categories. This process enables accurate provenance verification, ensuring the integrity of the seafood supply chain and aiding regulatory authorities in monitoring and enforcement.

CONCLUSIONS AND RECOMMENDATIONS

While the seafood industry holds immense potential for economic growth and food security, it also faces significant challenges related to food fraud. In today's world, where the food supply chain is highly complex and involves numerous actors from farm to plate, transparency is more critical than ever. Addressing these challenges requires a collaborative effort from all stakeholders and the application of scientifically robust analytical techniques to verify the authenticity of seafood products and ensure transparency throughout the supply chain.

To combat food fraud, stringent regulations and robust traceability systems are essential. Governments and industry stakeholders must work together to implement effective monitoring and enforcement mechanisms. This collaboration can help establish standards that ensure the integrity of seafood products and protect consumers from fraudulent practices. Additionally, consumer awareness and education play a crucial role in mitigating food fraud. By understanding the risks and knowing how to identify genuine products, consumers can make informed choices and support ethical practices within the industry.

Moreover, advancements in technology, such as isotopic and multi-elemental analyses, as well as machine learning, offer promising solutions for enhancing traceability and authenticity verification. These technologies can provide real-time data and insights, making it easier to track seafood from its origin to the final consumer. Ultimately, a multifaceted approach involving regulation, technology and consumer education is key to safeguarding the seafood industry against fraud and ensuring its sustainable growth.

CHAPTER 9
Conclusions

The impact of food fraud is significant, including the provision of unsafe or lower-quality products that can harm consumer health and undermine trust in the food industry and industry authorities. Although the incidence of food fraud varies by region, it is a global issue, and the aquatic sector faces unique challenges due to species diversity, different costs of production of wild versus farmed products, demand for processed products, and product perishability. Although food fraud affects all parts of the food value chain, studies have shown high rates of fraud towards the end, with one in five samples of aquatic products being mislabelled worldwide.

Food fraud can lead to food-safety and food-quality issues. For instance, species substitution and illegal harvest can hide food-safety hazards, leading to health consequences such as exposure to contaminants, exposure to veterinary drugs, food poisoning, and even death. Other issues, such as selling previously frozen products as fresh products, compromise quality and promote bacterial growth. In addition, there are nutritional differences between wild-caught and farmed fish, containing different fatty-acid profiles and total fat.

There are several tools for fighting food fraud. Among them are tools for verifying product traceability, which, when combined with a good understanding of fish taxonomy, are the most affordable tools to ensure the origin of the product and species identification. Tools of great relevance are national legislation and national and international standards, which are vital in defining acceptable products and practices.

International standards such as those developed by FAO and Codex Alimentarius are crucial in combating food fraud, and the ongoing work to develop Codex guidelines on the prevention and control of food fraud will provide additional solutions. Private food-safety standards, benchmarked by the GFSI, also play a significant role in setting standards for food supply chains that directly or indirectly support the fight against food fraud. These standards vary in detail and requirements for mitigation plans.

Lastly, it is important to highlight the relevant role of analytical tools in combating food fraud. Protein-based methods such as IEF, two-dimensional electrophoresis (2-DE), ELISA, HPLC and mass spectrometry (MS) are used for species identification but have limitations, such as being unsuitable for processed products and requiring preliminary selection protocols. DNA-based methods such as DNA barcoding, PCR, or microarrays offer higher specificity and sensitivity and are more effective for food authentication, even in highly processed products, but can be affected by PCR bias and fluctuating levels of mitochondrial DNA. Innovative DNA-based methods such as next-generation sequencing (NGS) technologies, including second-, third- and fourth-generation sequencing, can simultaneously sequence all DNA molecules in a sample. Applications such as metabarcoding and shotgun sequencing are used for seafood authentication. Metabarcoding combines NGS with DNA barcoding to analyse genetic variation, while shotgun sequencing avoids PCR bias and accurately quantifies the biological content of a mixture of food products.

The combination of traceability verification, an understanding of fish taxonomy, regulatory instruments, standards and analytical tools described in this report can significantly reduce fraud in the fisheries and aquaculture sector. Grounded in science-based policy, technological innovation and collaborative governance, these tools and mechanisms can help achieve authenticity and integrity within the sector.

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The global fisheries and aquaculture sector, producing over 185 million tonnes of aquatic products in 2022 and valued at USD 195 billion, faces growing vulnerability to food fraud. This complexity stems from the diversity of traded species (over 12 000) and the involvement of multiple inspection authorities across international supply chains, among other things. Common fraudulent practices include species substitution, mislabelling, adulteration, counterfeiting, and misrepresentation of origin or production methods. These actions, often economically motivated, pose serious risks to public health, consumer trust, and marine conservation.
The Food and Agriculture Organization of the United Nations (FAO) and the Joint FAO/IAEA Centre of Nuclear Techniqes in Food and Agriculture have worked together to provide an overview of the common food fraud cases in the aquatic sector and the associated health risks. The report resulting from this collaboration provides information on tools that can be used to fight food fraud for aquatic products, and international case studies illustrate the scope and impact of fraud. The report reviews regulatory frameworks as well as standards such as those set by Codex Alimentarius, FAO guidelines, and GFSI-benchmarked schemes, advocating for harmonized labelling, mandatory scientific names, and improved traceability. It emphasizes the role of consumer awareness and industry transparency in combating fraud.

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